RENAL FUNCTION

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JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Third Conference on Renal Function, I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program We are interested, first of all, in furthering knowledge about renal function and to this end the participants were brought together to exchange ideas, experiences, data, and methods In addition to this particular goal, however, there is a further, and perhaps more fundamental aim which is shared by all our conference groups This is the promotion of meaningful communication between scientific disciplines

The problem of communication between disciplines we feel to be a very real and a very urgent one, the most effective advancement of the whole of science being to a large extent dependent upon it Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another, channels must be established for the most relevant dissemination of this knowledge

The increasing realization that nature itself recognizes no boundaries makes it evident also that the continued isolation of the several branches of science is a serious obstacle to scientific progress Particularly is it so in medicine that the limited view through the lens of one discipline is no longer enough For example today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation At the other extreme medicine is certainly a social science and through mental health, must be concerned with economic and social questions The answer, then is not further fragmentation into increasingly isolated specialties disciplines and departments but the integration of science and scientific knowledge for the enrichment of all branches This integration we feel can be encouraged by providing opportunities for a multiprofessional approach to given topics

Although the fertility of the multidiscipline approach is recognized adequate provision is not made for it by our universities scientific societies and journals And perhaps the presence of other hindering factors must be admitted Partly semantic in nature

they may also to some degree be psychological Admittedly it is oftentimes difficult to accept data derived from methods with which one is unfamiliar By making free and informal discussion the central core of our meetings we hope to achieve an atmosphere which minimizes as much as possible these emotional barriers

Thus our meetings are in contrast to the usual *scientific* gatherings They are not designed to present neat solutions to tidy problems but to elicit provocative discussion of the difficulties which are being encountered in research and practice For this reason we ask that the presentations be relatively brief and that emphasis be placed on discussion as the heart of the meeting Our hope is that the participants will come prepared not to defend a single point of view but to take advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way that they would talk with their own colleagues in their own laboratories

We have now thirteen groups functioning under the Conference Program The following topics are covered adrenal cortex aging blood clotting cold injury connective tissues consciousness cybernetics infancy and childhood liver injury metabolic interrelations nerve impulse renal function and shock and circulatory homeostasis

When a new conference is organized the Chairman in consultation with the Foundation selects fifteen scientists to be the nucleus of the group and every effort is made to include representatives from all pertinent disciplines From time to time new members are added by the group to fill gaps in viewpoint or technique A limited number of guests are invited to attend each meeting but for the purpose of promoting full participation by all members and guests attendance at any meeting is limited to twenty five It is inevitable that in no topic can we possibly include more than a small fraction of the key investigators in the field and one of the difficulties in forming a group like this is that it is necessary to leave out so many people whom we would like to include

The transactions of these meetings are recorded and published This is done because the Foundation wishes to make current thinking in a field available to all those working in it and because it believes that conveying to those in other fields who are concerned with science for example government officials administrators etc the essential nature of scientific research is also an important problem in communication Logic is a vital aspect of science but equally essential is the intuitive or creative aspect Research is as creative as the painting of a portrait or the composing of a sym

phony Although logic is, of course, necessary in order to rearran to test, and to validate, research thrives on creativity which its source in unconscious, nonrational processes Unfortunate however, in the finished products which are presented to the wo through research reports this integral part of scientific endea is shriveled by the cold, white light of logic By preserving informality of our conferences in the published transactions, hope to give a truer picture of what actually goes on in the mi of scientists and of the role which creativity plays

FRANK FREMONT-SMITH, M D ,
Medical Direct

INTRODUCTORY REMARKS

ROBERT F PITTS
Chairman

I SHALL CONFINE my remarks largely to welcoming our members and guests and to acknowledging my indebtedness to them I am especially indebted to those who assisted me in arranging this program To all who are present my thanks for making this meeting possible And to those who are to open the discussions my special thanks for their willing acquiescence has made the task of the Chairman an easy one

This is for the benefit of those who have not attended previous sessions the third of our conferences on renal function The first dealt with renal tubular functions the second with the endocrine control of renal function more specifically with the control exerted by the adrenal cortex and posterior pituitary and this the third is to deal with physical factors in urine formation

There are two innovations which the Foundation has introduced this year which I think are highly laudable and which should increase the interest and value of these conferences The first is the inviting of a guest from abroad and we are very fortunate this year in having with us Dr Frank Winton from University College London The other is that the Foundation has invited representatives from each of the three major divisions of the Armed Forces We hope that the conference will be beneficial to them I am sure that their contributions to this discussion will be helpful to us

We shall proceed with the first of our discussions We are fortunate in having Dr John Pappenheimer to orient our thinking on the subject of filtration across capillary membranes Because there is no general agreement as to what is meant by filtration we are no less fortunate in having as a guest Dr Chinard a leader of the opposition We shall be most interested in the views of these two individuals for the help they will be in organizing our thoughts on the meaning as well as the mechanism of filtration

THE PASSAGE OF SUBSTANCES ACROSS CAPILLARY WALLS*

JOHN R PAPPENHEIMER
Department of Physiology Harvard Medical School

I SHALL TRY to present a picture of capillary permeability in terms of the number and dimensions of aqueous channels through the capillary wall which would explain observed permeability to lipid insoluble molecules ranging in size from water to hemoglobin The experimental work from which this picture developed was not carried out with kidneys but with a specialized preparation the isolated perfused hindlimb of the cat It is possible, however that the theories which have grown out of this work on peripheral capillaries may have application to the study of glomerular capillary filtration, peritubular capillary reabsorption, "back diffusion" across renal tubules, or other physical processes involved in the formation of urine Several new theories are involved, and I shall try to concentrate on describing the evidence for them without attempting to apply the results to the kidney, hoping, nevertheless, that such applications may emerge in the discussion

Membranes which restrict the passage of large molecules to about the same degree may offer very different resistances to the hydrodynamic flow of fluid through them In Table I are listed the filtration characteristics of a variety of membranes which have one property in common, namely, that they all retain 95 to 99 per cent albumin The term 'filtration' is perhaps ambiguous for by common usage in renal and capillary physiology 'filtration rate' has come to mean rate of hydrodynamic flow through the membrane rather than 'sieving' of molecules It is therefore important to define 'filtration constant" in precise terms

The hydrodynamic flow of fluid through porous media usually obeys the following law (Darcy's Law)

$$Q_f = \frac{K A_m \Delta P}{\eta \Delta x} \qquad [1]$$

where Q_f rate of flow (filtration), ml per sec
A_m = area of membrane, cm ^{2}

* The work presented for discussion in this paper was conducted in collaboration with E M Renkin A Soto Rivera L Borrero and S L. Eversole It has been supported by grants from the Life Insurance Medical Research Fund and the Eugene Higgins Trust

η = viscosity of filtrate, dyne sec per cm ^{2}
Δx = path length through medium, cm
ΔP = pressure drop across medium, dynes per cm ^{2}

The proportionality constant K has the dimensions of cm ^{2} and it is determined solely by the number and dimensions of the pores When used in connection with non-living systems, it is often called the "coefficient of permeability"(1) I have (perhaps misguidedly) called it the filtration constant since this term has been used previously in the physiological literature

TABLE I
Filtration Characteristics of Certain Membranes

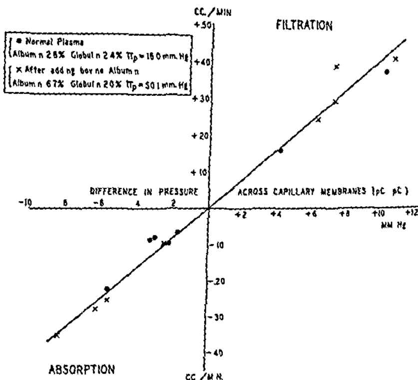
Membrane		Relative filtration constant cm x 10^{12}
1	Muscle capillary, cat	0 2
2.	Mesenteric capillaries, frog	5
3	Glomerular capillaries frog	19
4	Glomerular capillaries mammals	22
5	Collodion membrane, 0 3 x 10^4 cm thick and of pore diameter (110 Å) sufficient to retain 95 per cent of serum albumin*	780

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In dealing with living capillaries, we can, in certain instances, measure experimentally all the quantities required to define K except the path length, Δx, through the capillary wall It is therefore convenient to consider a relative filtration constant K′ which includes Δx and is defined by $K' = K/\Delta x$ The relative filtration constant will then have the dimensions of cm as shown in Table I To date, the relative filtration constant has been measured in two types of capillaries (*a*) the individual capillaries of the frog's mesentery(3), and (*b*) the muscle capillaries in the perfused hindlimb(4,5) In both preparations the rate of filtration has been shown to be accurately proportional to the pressure difference across the membrane Of course if the filtering fluid contains solute molecules to which the membrane is entirely or partially impermeable, there will be osmotic pressure gradients across the mem

* This value is calculated from the data of Elford and Ferry(2) on the assumption that relative filtration constant varies inversely with membrane thickness The actual thicknesses used for these measurements were 50 microns or more

brane which must be considered in computing the pressure difference During filtration of normal plasma across capillary membranes at normal physiological pressures, the only molecules which establish appreciable osmotic gradients are the proteins, and, consequently, the rate of net filtration or net absorption is proportional to the difference between mean capillary hydrostatic pressure and the protein osmotic pressure Figure 1 shows that



[illegible] The rate of fluid exchange is [illegible] pressure in the [illegible] posing filtration [illegible] bsolute value of these quantities Reprinted by permission from Pappenheimer J R and Soto-Rivera A *Am J Physiol* 152 471 (1948)

this is true in the hindlimb perfused with protein solutions of osmotic pressure ranging from 16 to 50 mm Hg The rate of net hydrodynamic flow across the capillaries was proportional *to the difference* between the mean capillary hydrostatic pressure and

the effective protein osmotic pressure When converted to c g s units divided by the capillary surface area (determined histo logically) and multiplied by the viscosity of the filtrate the slope of the line shown in Figure 1 is numerically equal to the relative filtration constant listed in Table I Data similar to those shown in Figure 1 have been obtained from about one hundred cat hindlimb preparations perfused with whole blood or protein solutions at temperatures ranging from 10 to 45°C At low temperatures the net rate of flow of fluid across the membranes per unit pressure difference is decreased approximately in proportion to the increase of viscosity of water as shown in Table II and predicted from Equation [1] Figure 1 and Table II taken together provide evidence that we are in fact dealing with viscous flow through aqueous channels

TABLE II

Effect of Temperature on Resistance to Hydrodynamic Flow and Molecular Diffusion Through Muscle Capillaries

	36 ± ° C	10 ± °°C	Rat o
Filtration coefficient K_f (Av of 15 experiments) ml /min /mm Hg/ 100 g muscle	0 0146	0 0083	$K_f^{36°} / K_f^{10°} = 1\ 76$
Average diffusion ½ time across capillaries minutes	NaCl 5 3 Sucrose 7 3	9 7 12	$½T_{10°} / ½T_{36°}$ = 1 8 = 1 6
Viscosity of water centipoises	0 7085	1 308	$\frac{\eta 10°}{\eta 36°} = 1\ 85$

Let us now return to Table I The figures given for frogs mesen teric and cat hindlimb capillaries come directly from experimentally measured quantities In the case of the remaining membranes some of the primary quantities required for calculating relative filtration constant have not yet been measured experimentally and we have had to make some assumptions The detailed assumptions involved may be found in Reference 5 It is interesting that our guess for glomerular membranes leads to about the same value as that recently proposed by Verney and Rushton(6) who used somewhat different assumptions in performing their calculations Although we must regard some of the values given in Table I as little more than "orders of magnitude they nevertheless point to an

capillaries and about 4000 fold more resistance than collodion membrane of comparable thickness and permeability to protein On the pore theory of capillary permeability, we would infer that the pores in muscle capillaries are 4000 times less numerous per unit surface area than in collodion membrane of comparable pore size The collective pore area in such a collodion membrane comprises about 60 per cent of the membrane surface(2) so that we may guess that only 60/4000 or 0 015 per cent of the muscle capillary contains pores for the filtration of fluid The corresponding figure for the glomerular capillaries would be 1 to 2 per cent Evidence in support of this conclusion comes from the study of molecular diffusion through the capillary walls, and to this study I now propose to turn

If an inert, lipoid insoluble, molecular species is added suddenly to arterial blood supplying a tissue, there results an osmotic disturbance consisting of two related processes (*a*) the added molecules tend to diffuse from the plasma, across the capillary membranes, to the interstitial fluid, (*b*) the concentration gradient of added molecules across the capillary membranes results in osmotic withdrawal of fluid from the interstitial compartment into the capillary blood Both processes continue until the concentration difference across the capillary wall approaches zero as a result of loss of molecules from blood to extravascular fluid and dilution of the blood by the absorbed interstitial fluid The rate at which these processes take place depends, in part, on the hindrance to diffusion of the added molecules through the capillary walls

In the isolated perfused hindlimb preparation, the osmotic withdrawal of fluid during such an osmotic transient may be prevented by raising the mean hydrostatic pressure in the capillaries by known amounts just sufficient to maintain the limb at constant weight(4) Under these conditions, the increment of mean capillary pressure is a measure of the partial osmotic pressure (or diffusion pressure) exerted by the added molecules across the capillary membranes Figure 2A shows the time course of such osmotic transients for urea and for inulin diffusing across the capillary membranes in the hindlimb of a cat Throughout each transient, the mean capillary pressure was continually adjusted by varying the venous pressure so that the leg remained at constant weight (isogravimetric state)

Pitts How do you avoid the difficulties of changes in volume of your vascular system?

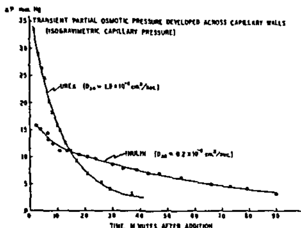


Fig 2a

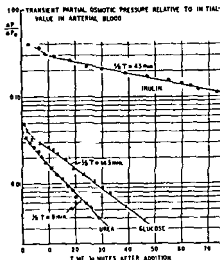


Fig 2b
EXPONENTIAL RATES

OSMOTIC TRANSIENTS IN CAPILLARY CIRCULATION

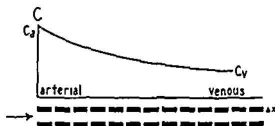
FIGURE 2 Reprinted by permission from Pappenheimer J R Renkin E M and Borrero L Am J Physiol 167, 13 (1951)

Pappenheimer It is quite true that the large changes of venous pressure required to maintain the isogravimetric state during an osmotic transient cause changes of vascular volume which tend to

mask the changes of weight due to net filtration or net absorption However the changes of vascular volume following a sudden change of venous pressure are almost instantaneous in comparison to the slow diffusion process Also the pressure volume curve of the venous system is hyperbolic so that at high venous pressures such as those required to balance the initial osmotic effects considerable changes of venous pressure are required to produce significant changes of vascular volume Nevertheless the problem is an annoying one particularly in the first stages of an osmotic transient requiring an initial large rise of venous pressure It has been largely *avoided by raising the venous pressure to approximately* the correct value *before* the test molecules are added the correct peak value being determined approximately from previous experience In this case rapid net filtration is occurring before the test molecules are added and the initial osmotic effect of the added solute is to bring the preparation back to the isogravimetric state With this method measurements can be made within three to four minutes of adding the test substance this time being *required for adequate mixing in the perfusion system*

The osmotic transients so measured decline exponentially with time as shown in Figure 2B and the time constant of exponential decay varies inversely with molecular size This method of depicting the results has not however proved valuable except in one respect It will be noted that extrapolation of the curves of Figure 2B to zero time yield osmotic pressures which are only small fractions of the theoretical van t Hoff pressures predicted from the concentrations of the test molecules in *arterial* plasma Thus urea exerted only 4 per cent glucose 6 per cent and inulin 40 per cent of the initial van t Hoff pressure in arterial plasma This is an important point because concentration in arterial plasma has been widely used as a measure of concentration in capillaries for calculations relating to transcapillary exchange rates of tracer molecules injected into the vascular system The reason why concentration in arterial plasma is such a poor measure of that in capillaries is easily understood if measurements are made of arteriovenous concentration differences Figure 3 shows the arteriovenous difference transient for D_2O following its addition to the perfused hindlimb preparation Clearly the mean capillary concentration is very much less than the arterial concentration and there must be a large gradient of concentration along the length of the capillary

Data of the type illustrated in Figure 2 may be combined with that of Figure 3 to yield information concerning the nature of the capillary wall considered as a barrier to diffusion Figure 4 illus



$$\text{I} \quad \frac{dm}{dt} = Q_p (C_a - C_v)$$

$$\text{II} \quad \frac{dm}{dt} = D\,A\,\frac{\overline{\Delta c}}{\Delta X}$$

$$\text{III.} \quad P = RT\,\overline{\Delta c}$$

$$\therefore \frac{A}{\Delta X} = \frac{Q_p(C_a - C_v)\,RT}{D \times P}$$

Figure 4

is going in and what is coming out as stated algebraically by equation I of Figure 4 Equation II in Figure 4 states that if the test molecules are leaving the vascular system by a process of diffusion through aqueous channels, then the rate will be given by Fick's Law It is important to call attention to two special features of equation II (*a*) if D is taken as the *free* diffusion coefficient of the test molecules in water, then A may be a virtual area referring to that area required for *free* diffusion at the rate dm/dt, (b) the mean concentration difference $\overline{\Delta c}$ across the capillary wall is the resultant of several unknown factors Thus, the concentration within the capillary decreases along the capillary length, as suggested by the curve linking the arterial with the venous concentration The form of this curve at any moment is unknown Also, the concentration outside the capillary wall is unknown and may be non uniform The important point is that *the same unknown* $\overline{\Delta c}$ *which provides the gradient for diffusion is also responsible for the observed osmotic pressure, P, defined by equation III* This takes account also of different concentration gradients in different capillaries

The three equations of Figure 4 may now be combined to eliminate dm/dt and $\overline{\Delta}_r$, thus solving for A/Δ x in terms of experimentally measurable quantities Hence

$$\frac{A}{\Delta x} = \frac{Q_p\ (C_a - C_v)\ RT}{D \times P} \qquad [2]$$

If equation [2] is correct, then A Δx is a geometrical property of the capillary wall and should be independent of any changes in the terms on the right hand side of the equation In any given experiment in which the blood flow and temperature are maintained constant, the only variables are the A V difference in concentration and the osmotic transient, and we can predict that their ratio must remain constant Experimental verification of this prediction is illustrated in Figures 5A and 5B which show simul-

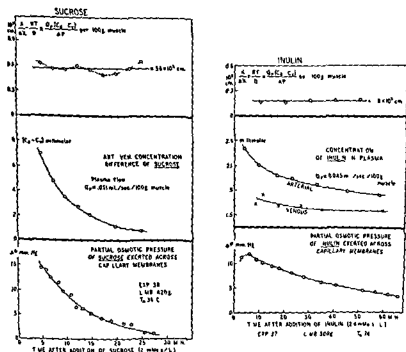


FIGURE 5 Reprinted by permission from Pappenheimer J R Renkin E M and Borrero L. *Am J Physiol* 167, 13 (1951)

taneously measured osmotic and A-V difference transients for the case of sucrose and inulin It is clear that in this experiment the ratio of A-V difference to osmotic pressure remained approximately

constant throughout the transient When multiplied by Q_i and R T/D, the ratio should be numerically equal to the area per unit length in the capillary walls which is available for the diffusion of these substances Similar results have been obtained with a variety of molecules and will be considered in a moment Further evidence in support of equation [2] comes from studying the effects of changing the blood flow If $A/\Delta x$ is a geometrical property of the capillary wall, its value must be independent of the flow rate Figure 6 shows the effects of varying the blood flow (by varying the arterial perfusion pressure) on the osmotic and A-V difference transients in the case of raffinose It is seen that at low blood flow the A V difference is greatly increased and by just such an amount that the calculated value for $A/\Delta x$ remains constant

Dock Does that mean that the area does not change as the capillaries are dilated by the rise of pressure necessary to produce greater flow?

Pappenheimer In the experiment of Figure 6 there was in fact no change of mean capillary pressure When the arterial pressure was increased to produce the high blood flow, the venous pressure was simultaneously decreased to keep the limb isogravimetric (i e maintain constant mean capillary pressure) Nevertheless, even if the capillary pressure had been raised (within physiological limits), *there would, I think, have been no detectable change of area* The reason for this supposition is given in Figure 1 which shows that the rate of fluid exchange is proportional to the *difference* in pressure across the capillary wall but is independent of the *absolute* hydrostatic pressure over a wide range I think the mechanics involved in this constancy of area with change of capillary pressure may possibly be found in Burton's recent formulation of the relations between pressure wall tension, and tube radius(7) When the radius of a vessel is small, very large pressures indeed are required to stretch the wall To be sure capillaries will dilate under the influence of metabolites, pharmacological agents etc and we have studied the effects of several drugs, both dilator and constrictor, from this point of view Although these agents alter the resistance to blood flow over a ten fold range, they do not affect the filtration coefficient or osmotic transients by significant amounts I suspect that in the isolated preparation the capillaries are already dilated and the effects of epinephrine, urethane acetylcholine, pitressin, chloral hydrate, etc , are exerted principally on the arterioles and venules But that is another story

Let us consider now that our theories thus far are correct and that we can measure the area per unit path length in the capillary

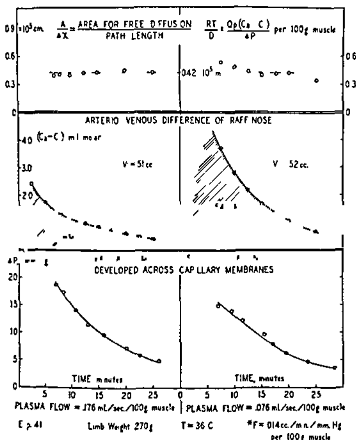


FIGURE 6 Diffusion of raffinose at two blood flows from capillaries in hindlimb of cat Exper 41 limb weight 270 gm $T = 36°C$ $K_f = 014$ ml per min per mm Hg per 100 gm muscle The blood flow was altered by varying the arterial perfusion pressure The same quantity of raffinose was added to the perfusion reservoir at each of the two flows Note that the time course of the osmotic transient was relatively unaffected by the flow rate whereas the arteriovenous difference was increased at low flow and by an amount such that the calculated area per unit path length was unaltered as would be expected of a geometrical property of the capillary wall

flow
th n
The
/C

here M is the amount which diffused and C_a is the measured equilibrium concentration It was 51 ± 1 ml or 19 per cent of the limb weight at both flows

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walls required to explain the observed diffusion rate of any test molecular species What do the results show?

It is evident from Figures 5A and 5B that the area available for free diffusion of inulin through the capillary walls is considerably less than that for glucose In Figure 7 are plotted, as a

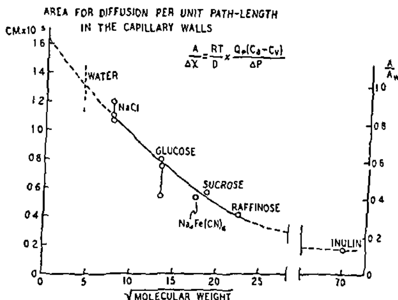


FIGURE 7 Modified from a figure appearing in Pappenheimer J R Renkin E M and Borrero L *Am J Physiol* 167, 13 (1951) and reprinted by permission

function of molecular weight, the free diffusion areas per unit path length available to six molecular species Since the path length *is presumably the same for each species, it follows that the* area for free" diffusion becomes progressively restricted with increasing molecular weight What absolute values of area are involved? From Figure 7, the area per unit path length for *NaCl* is 1 1 x 10^5 cm in 100 grams of muscle, and extrapolation to a molecule the size of water yields only a slightly greater value Thus, for a molecule the size of water, A = 1 3 x $10^5 \Delta x$ cm ^{2} We do not know the path length, Δx, but it can hardly be more than *one micron because the capillaries in muscle* are often only five microns in outside diameter(8) A high estimate for the diffusion area for a molecule the size of water would, therefore, be 1 3 x 10^5 x 10^{-4} or 13 cm ^{2} The diffusion areas *available to the* other molecular species shown in Figure 7 are less than this as

indicated on the scale relative to water (A A$_w$) on the right hand ordinate

This estimate for diffusion area may now be compared with the total surface area of the capillaries in 100 grams of perfused muscle. A precise figure for capillary surface area is hard to obtain. Usual histological techniques involving perfusion with India ink followed by fixation paraffin imbedding and capillary counts have led previous investigators to estimates ranging from 1 75 to 8 2 M^2 per 100 grams of muscle. Any value above 4 M^2 is however improbably high because it would imply a capillary volume of at least 4 per cent of the muscle weight and the total blood volume in muscle is no more than this. Our own estimates in the perfused hindlimb preparation are lower than this (about 0 7 M^2 per 100 grams) possibly because we have used frozen sections which avoid shrinkage and this results in fewer capillary counts per mm^2. Comparison of our estimate for diffusion area (less than 13 cm^2) with our estimate for capillary surface area (approximately 7 000 cm^2) leads us to the conclusion that the fractional pore area in the capillary walls available to a molecule the size of water is less than 0 2 per cent. This small fraction is of the same order as that estimated above (see page 16) from the hydrodynamic resistance to flow offered by the muscle capillaries in comparison with collodion membranes of comparable thickness and pore size. It lends support to the view that the diffusion of small lipid insoluble molecules takes place through the same channels as hydrodynamic flow and that these channels comprise only a minute portion of the total capillary surface

Fremont Smith With respect to water are you working on the basic assumption that at equilibrium no water exchange is taking place?

Pappenheimer In the isogravimetric state there is almost no *net* exchange of water across the capillaries. I say "almost" because there is actually a very small net flow of fluid into the tissues to balance the evaporative losses from the surface of the perfused limb. However two types of steady state exchange of water molecules across the capillaries are undoubtedly occurring. The first is hydrodynamic flow out of capillaries or portions of capillaries where the hydrostatic pressure exceeds the effective osmotic pressure. This is balanced by an equal rate of flow into capillaries or portions of capillaries where the hydrostatic pressure is less than the effective osmotic pressure. This rate may be estimated from the filtration coefficient of the capillaries (Figure 1) and the hydrostatic pressure drop along the capillary length (about 10 mm Hg)

It is of the order of 0 0001 ml per sec per 100 grams of muscle The energy for this exchange comes from the heart and is dissipated in overcoming the frictional resistance to flow offered by the pores The second type of steady state exchange consists of diffusion of water molecules back and forth through the capillary pores This rate is prodigiously large even though the total pore area is so small Thus water in a plasma concentration of 92 grams per 100 ml would be expected from Fick's law to diffuse at the rate of about 3 grams per second through capillary pores 1 micron thick and with a total diffusion area of 13 cm^2 Actually the rate of diffusion outward from plasma to interstitial fluid would be about 99/92 greater than this owing to the increased thermodynamic activity of plasma water molecules resulting from the hydrostatic pressure within the capillary required to balance the greater concentration of water on the tissue fluid side of the membrane (another way of talking about protein osmotic pressure) This is a point which I am sure Dr Chinard will bring out precisely in his presentation The energy for this diffusion back and forth through the capillary pores comes from the kinetic energy of the water molecules I need hardly emphasize that the diffusion rate in both directions is so large because of the shor path length involved Owing to the square law, the velocity diffusion in microscopic and ultramicroscopic systems becomes matter of milliseconds in comparison with hours and days macroscopic systems This point is of utmost importance in c paring artificial membranes with capillaries

Tremont Smith Is it possible that diffusion of water in both directions may take place through areas different from the area you have measured? Are you not measuring only the area available for net change? The area for "in net" change might be 10 000 times greater or any other unknown figure might it not?

Pappenheimer That is an interesting idea but I cannot conceive of pores which could distinguish between the two The fact that the area required to explain the observed resistance to hydrodynamic flow turns out to be about the same as that for diffusion may perhaps be considered evidence that there are no other large areas involved for the passage of water In the next stages of the analysis I am going to show that this small area for both flow and diffusion leads to a reasonable solution for pore dimensions and an explanation of the restricted diffusion areas available for a variety of solute molecules Perhaps this will provide further information in answer to your question

I am now going to derive the dimensions of any given pore

geometry In order to illustrate this derivation let us consider the simple case of uniform cylindrical pores We shall assume that hydrodynamic flow through these pores may be described by Poiseuille's law with a small correction which allows for the fact that the pore area available for water is slightly less than that available to a molecule of zero molecular weight (e g infinitely small compared to the true pore size See Figure 7)

$$\frac{Q_f}{\Delta p} = \frac{N\pi r^4}{8\eta\Delta x} = \frac{A_f\, r^2}{\Delta x\; 8\eta} = K_f \qquad [3]$$

Where $\frac{Q_f}{\Delta p}$ = flow per unit pressure difference across the capillary walls which is measurable experimentally as shown in Figure 1 and is designated by K_f The radius of the pore is r and A_f is the collective pore area available for filtration Solving for r we have

$$r = \sqrt{\frac{8\eta K_f}{A_f/\Delta x}} \qquad [4]$$

But if the area for filtration is the same as that for diffusion of a molecule the size of water then we know the value of $A_f\ \Delta x$ from the results shown in Figure 7 Typical values for insertion into equation [4] are $\eta = 0.007$ poises $K_f = 2.1 \times 10^{-7}$ ml per sec per dyne per cm^{-2} $A_f/\Delta x = 1.3 \times 10^5$ cm Whence $r = 30 \times 10^{-8}$ cm

This result means that uniform pores of radius 30 Ångstrom Units and of collective area per unit path length 1.3 x 10^5 cm would offer the observed hydrodynamic resistance to flow and at the same time satisfy the predicted diffusion rate for a molecule the size of water The same methods may be employed to calculate the pore radii in any given distribution of cylindrical pores or to solve for the dimensions of any other given pore geometry There are in fact an infinite number of distributions of pore sizes or geometries or their combination which will result in the observed hydrodynamic resistance to flow and diffusion rate for a molecule the size of water

In order to go further we must consider our remaining data namely the restricted diffusion areas available for the larger molecules shown in Figure 7 On first thought one might suppose that the restricted diffusion area for a molecule such as sucrose (e g 44 per cent of that for a molecule the size of water as shown in Figure 7) would be simply explained by assuming that 56 per cent of the pores were smaller than the smallest dimension of sucrose and 44 per cent were larger This simple explanation must however be discarded because the smallest dimension of sucrose

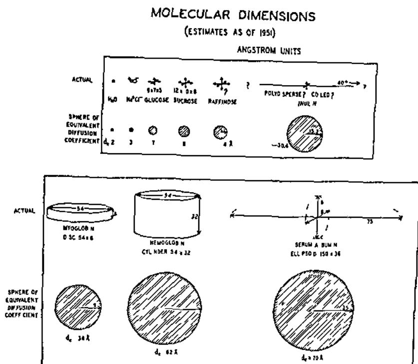


FIGURE 8

is 8 Å (Figure 8) and if 56 per cent of the pores were smaller than this, then all the remaining pores would have to be larger than 100 Å in diameter in order to satisfy the observed hydrodynamic resistance to flow Such a distribution would not satisfy the results for the remaining molecules We have to ask, therefore, whether the reduced diffusion areas available to molecules larger than water may be explained, at least in part by restriction to diffusion through uniform pores The following considerations suggest that severe restriction to diffusion may be expected when the radius of the diffusing molecule is comparable with the dimensions of the pore (*a*) The probability of a molecule entering a pore without striking the edge of the opening will depend on the area of the molecule relative to the area of the pore For the case of spherical molecules entering cylindrical pores, the free area for penetration will be $A = \pi(r - a)^2$ where r is the radius of the pore and *a* that of the molecule Relative to the area of the pore, the free area for penetration becomes $(1 - a/r)^2$

$$\frac{A}{A_w} = (1 - a/r)^2 \qquad [5]$$

(*b*) Once the molecule enters the pore its progress by diffusion will be retarded as a result of viscous drag at the stationary walls of the pore This factor has been studied quantitatively in connection with corrections to Stokes law in systems where particle size is comparable with the size of the sedimentation chamber For the case of spherical particles sedimenting in cylindrical tubes this correction has been given by Ladenburg(9) in the form $1/(1 + 2.4\ a/r)$ An analogous correction has been shown by Westgren (10) to apply to ultramicroscopic particles sedimenting in a wedge shaped container Westgren utilized the Tyndall effect to follow the rate of sedimentation of the suspended particles

Combining these factors which may restrict the rate of diffusion of spherical molecules through cylindrical pores we have

$$\frac{D \text{ restricted}}{D \text{ free}} = \frac{(1 - a/r)}{1 + 2.4\ a/r} \qquad [6]$$

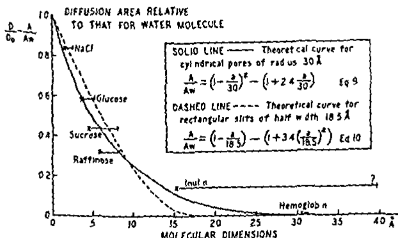


FIGURE 9. Comparison of theoretical with experimental values for restriction to ... of ... d ... se ... raffinose ... d with the ... of 18.5 Å ... to flow of ... length for a ... slits as the more likely pore geometry but more exact data with nearly spherical molecules wo... inte... mo... thr... complicated by some degree of polydispersion. Reprinted by permission ... Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M., *Am. J. Physiol.* 167, 13 (1951)

This theoretical restriction to diffusion is plotted in Figure 9 for the case of uniform cylindrical pores of radius 30 Å and compared with the observed restrictions to diffusion offered by the capillaries to the molecules we have studied so far Unfortunately none of the test molecules is actually spherical and there is therefore doubt as to the proper dimensions to use As a first approximation we have used the Einstein Stokes radius of the molecule e g the radius of a sphere of equivalent free diffusion coefficient but the longest molecular dimension is also depicted in Figure 9 as a horizontal bar Current estimates of these molecular dimensions are summarized in Figure 8 Inspection of Figure 9 shows that uniform cylindrical pores of radius 30 Å will account reasonably well for the observed restrictions to diffusion of the lipid insoluble molecules thus far studied For the case of a long chain molecule such as inulin the Einstein Stokes diffusion radius rather than the longest molecular dimension appears to be the important dimension to consider However we cannot trust this result too much because inulin may be polydisperse and it is possible that the observed area per unit path length available to inulin may be the mean of the areas available to two or more components

Chinard Ingelman(11) has indicated that a preparation of inulin studied in the ultracentrifuge was polydisperse No data were given The extent of the polydispersity of that preparation is not known nor do studies appear to have been made on material that is used clinically in this country

Pappenheimer Certainly the restriction to diffusion of mono disperse polymers of various shapes should now be investigated with the new methods both in capillaries and in artificial mem brane systems The results may be expected to provide information about the shape of the capillary pores and would be important also in designing molecules for use as plasma extenders

The fact that uniform cylindrical pores of radius 30 Å will account for the hindlimb data does not exclude other pore geometries or a limited distribution of pore sizes One alternative geometry namely rectangular slits of width 37 Å has already been shown to account for the facts as successfully as do cylin drical pores (Figure 9) However the data are not consistent with a broad distribution of pore sizes for reasons which have already been given semi quantitatively (see page 27) A Gaussian distribu tion of pore radii about a mean radius of 24 Å with a standard deviation of 12 Å is the broadest distribution consistent with our

observed hydrodynamic resistance to flow, with area per unit path length for a molecule the size of water and with restrictions to the diffusion of larger molecules

I have now presented most of the evidence and the theories which go to make up our present picture of the permeability (to water and lipid insoluble molecules) of capillaries in the muscles of perfused cat hindlimbs We might summarize this picture as follows

1) In 100 grams of perfused muscle there is a total capillary surface of about 7,000 cm.^{2} which is roughly the same as the glomerular capillary surface in one human kidney(12) The hydrodynamic resistance to flow through the walls of these muscle capillaries is such that a pressure difference of about 60 mm Hg is required to cause a filtration rate of 1 ml per minute This resistance is roughly 100 fold that of glomerular capillaries and 4,000 fold that of a collodion membrane of comparable thickness and permeability to protein, a fact which suggests that only a minute fraction of the capillary surface is available for hydrodynamic flow (filtration)

2) New methods developed for the study of diffusion rates indicate that the area available for the diffusion of a molecule the size of water is less than 13 cm ^{2} in the capillaries of 100 grams of muscle Both hydrodynamic flow and diffusion rates are slowed at low temperatures in proportion to the temperature coefficient of viscosity of water, a fact which suggests that aqueous channels are involved

3) Combination of diffusion data with hydrodynamic flow data allows a numerical solution for the number and dimensions of pores of any given geometry Uniform cylindrical pores of radius 30 Ångstrom Units and collective area per unit path length 1 3 x 10^5cm in 100 grams of muscle would form one type of pore system resulting in the observed resistance to flow and area for diffusion A population density of about 2 x 10^9 such pores per cm ^{2} of capillary wall would be required Other pore geometries such as uniform rectangular slits 37 Å wide and of collective length about 10 times that of the capillaries, would also account for the results

4) A theory has been developed to account for restriction to diffusion of molecules through pores of the above dimensions The theory agrees reasonably well with the facts and suggests that the system is relatively isoporous

Fremont-Smith Will you now distinguish sharply between filtration and diffusion?

Pappenheimer I have been using filtration rate in the hydrodynamic flow through the membranes The energy process is that required to overcome the viscous resistance passing through the membrane If a concentration differe across the membrane as a result of molecular sieving energy required to maintain flow against this concentra ference must be subtracted from the total I think it is cl Figure 1 that filtration rate in this sense is independen concentration gradient of protein across the membranes ' of hydrodynamic flow depends only upon the *difference* hydrostatic and effective osmotic pressures If the effective pressure within the capillaries exceeds the mean hydrosta sure by 10 mm Hg then there will be a net flow of intersti into the capillaries at precisely the same rate as that whicl out of the capillaries when the mean hydrostatic pressure *the effective osmotic pressure by 10 mm Hg When flow* into the capillaries it is called *absorption* when flow occur the capillaries it is called *filtration* This terminology ma poor one but it is widely used in renal and capillary phy Perhaps we should always refer to hydrodynamic flow in o avoid ambiguities If there were no concentration gradients the membranes then this same rate of hydrodynamic flow occur when the mean capillary hydrostatic pressure is 10 m

Diffusion on the other hand is independent of hydrod flow The driving pressure is due to the concentration gr and the energy is dissipated in overcoming the viscous resi of the solvent to the kinetic movements of the solute mol This is the basis of Einstein s derivation of Fick s law of di from kinetic theory Diffusion may occur rapidly in the dir opposite to that of hydrodynamic flow This is often the c the living capillaries A good example is the excretion of PA the mammalian kidney PAH passes rapidly by diffusion th the peritubular capillaries to the tubule cells in spite of the that there is a hydrodynamic flow (peritubular capillary ab tion) of about 120 ml per min in the opposite direction thr the same capillaries Conversely the rate at which small mole diffuse through the capillaries of the perfused hindlimb is appreciably accelerated by rapid net filtration caused by a venous pressure

The quantitative relations between transfer of substance diffusion as compared to filtration may be derived as follows

of inulin and molecules larger than inulin is sufficiently great to allow such concentration gradients during extremely rapid filtration These may be detected either chemically or osmotically as described in Reference 5

Selkurt What would be the effect of combining certain molecules in this situation? Wouldn t you expect, along the lines of what you are saying that that would also impede diffusion, if you think in terms of competition of these molecules for the pore? Have you analyzed any mixtures in terms of figuring out these diffusions?

Pappenheimer We have not investigated the effects of adding two or more lipid insoluble molecular species simultaneously I would suspect that they would not interfere with one another appreciably because the number of solute molecules involved would be so small compared to the number of water molecules

Winton There is a point that I am not clear about In your filtration equation, the A for the molecule, or A_w, might be affected by the presence of proteins if proteins are layered on the membrane That would impede the transfer, would it not?

Pappenheimer Yes, it would, in fact I think it does If all proteins are removed (e g perfusion with Ringer's solution), the filtration constant is greatly increased and the diffusion rates of small molecules are greatly increased also This is a reversible phenomenon addition of sufficient protein restores the normal permeability of the membranes One therefore, suspects that interaction between protein and capillary wall may be a factor in determining pore size Much work remains to be done along this line

Chinard You have pores which are holes in a presumably solid plate and you have hydraulic flow through these pores of molecular dimensions Where does the separation of protein take place?

Pappenheimer A few of the protein molecules will fall within a radius $(r - a)$ These molecules will slip through but all the rest go right around the circulation

Chinard Do the proteins pile up over the holes in process of filtration? I can't visualize the physical process involved here

Pappenheimer If they are too big to go through, then the area available by the formula as well as by the experiment is zero $(r - a) = 0$

Chinard I am talking about the restriction that protein might put on the passage as simple plugs

Berliner They will reduce the value of A and are included in the equation The circumstances under which A is determined is such that the effect of protein on the area available for movement is automatically incorporated

Pappenheimer. Yes, I think those go to make up A

Chinard I see

Pappenheimer But there I am on much more tenuous ground, really.

Thorn Is it true that you have observed no change in diffusion rate with these changes in pressure and that you are assuming that the membrane permeability stays the same?

Pappenheimer Yes

Thorn But do we have a right to assume that membrane permeability stays the same when we start the experiment with a relatively poor blood flow and end the experiment with a rapid blood flow?

Pappenheimer The answer to this really belongs to that part of the story which was published in 1948(4) The way these things are done is to start with an exceedingly high blood flow and then go down to the lowest you can get away with and still have the hindlimb in good condition In a typical preparation, the range usually covers from about 50 ml per min to 8 ml per min Extrapolation to zero flow then yields the isogravimetric capillary pressure which has been shown to equal the effective osmotic pressure across the capillary membranes In the steady state of fluid exchange, this pressure is about 95 per cent of the protein osmotic pressure as measured *in vitro*(4)

Fremont Smith Haven't you made a basic assumption all the way along that there is no temperature gradient across your capillary membrane

Pappenheimer Yes, but there is this about it if you put in a substance which does not go through the membrane such as hemoglobin or serum albumin, you get very nearly the theoretical van't Hoff pressure exerted across the capillary membranes

Fremont-Smith You are justifying the assumption?

Pappenheimer Exactly However, we have played with changes in temperature By suddenly changing the temperature of arterial blood supplying the preparation, perhaps from 40 to 10°C, one can observe enormous osmotic effects They are extremely complicated, however, because there are vascular reactions as well(13), so I can't say much about the non-steady-state of temperature Once a new steady-state is reached, the theoretical protein pressure is again measurable Filtration and diffusion velocities are, however, slowed at low temperatures in proportion to change in viscosity of water

Dock Do you want to say anything about lipid solubility here?

Pappenheimer The permeability of the capillaries to lipid soluble molecules appears to be quite different from that to the lipid insoluble molecules discussed so far About two years ago, when the results first indicated that such a small capillary area is involved in the transport of lipid insoluble molecules, Roughton pointed out that this small area would not account for the observed rate of transport of oxygen and CO_2 across the capillaries The answer to this discrepancy appears to be concerned with lipid solubility Both these molecules are more soluble in olive oil than in water We have since found that such molecules, even in high osmotic activity, traverse the capillaries so rapidly that no osmotic transients are detectable Glycerol behaves as predicted, but if you substitute acetate groups getting larger molecules of progressively greater solubilities in olive oil, you get less and less osmotic pressure(14)

Pitts How do your data bear on the problem of the site of passage of water and electrolytes through the capillary wall, do these materials pass through the intercellular cement or through the cell membrane? Are the cells present merely to hold the intercellular cement in place or do they function more actively as the matrix supporting and separating the pores?

Pappenheimer Needless to say, our experiments tell us nothing directly about the anatomical location of transfer sites It is tempting to speculate from the results, however, that the small area available for the exchange of lipid insoluble molecules is localized in intercellular regions which contain aqueous channels of the dimensions we have deduced, whereas lipid soluble molecules dissolve in the plasma membranes of the endothelial cells themselves and are thus capable of utilizing the entire capillary surface We, therefore, think of two types of capillary permeability, one of these is similar to that of cells in general, that is a high degree of cellular permeability to lipid soluble molecules and a relatively low degree of cellular permeability to water and electrolytes To this must be added the permeability resulting from specialized aqueous channels between cells Perhaps these channels are formed by a gel structure or a fibrous structure We do not know We do know that it presents the same barrier to hydrodynamic flow and diffusion of solutes as would uniform cylindrical pores of radius 30 Å and area per unit path length 1.3×10^5cm per 100 grams of muscle

Bott May I ask a question which is of practical importance to me? If you increase protein (such as hemoglobin) concentration in the plasma why does the proportion that comes across the membrance increase?(15,16)

Shock Hemoglobin clearances on humans have been done in our laboratory by Dr McDonald and Dr Miller(17) Our results show that as the amount of hemoglobin in the plasma is increased the amount of hemoglobin excreted also increases The amount of hemoglobin excreted per unit of glomerular filtrate goes up linearly with increasing concentrations of hemoglobin in the plasma (Figure 10)

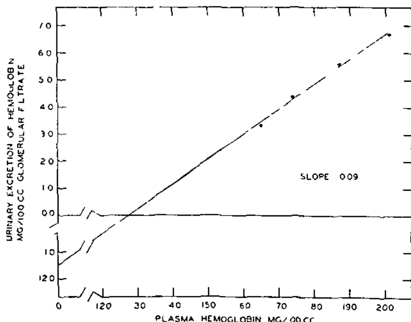


FIGURE 10 The relationship between plasma hemoglobin concentration and the urinary excretion of hemoglobin per 100 ml of glomerular filtrate The x intercept of the linear function indicates the theoretical renal threshold for hemoglobin The y intercept indicates the tubular recovery of hemoglobin in mg per 100 ml of filtrate The subject received 8 5 gm hemoglobin at a rate of 60 mg per min Reprinted by permission from McDonald R K Miller J H and Roach F B *J Clin Investigation* 30 1042 (1951)

Dock These results are similar to those published by Brandt(18)

Berliner But the slope of the relationship of hemoglobin excretion to plasma hemoglobin concentration becomes the filtration rate of hemoglobin does it not?

Shock The slope of the plot of urinary excretion per unit of glomerular filtrate against plasma hemoglobin concentration indicates the permeability of the glomeruli to hemoglobin relative to inulin

Berliner It suggests that the concentration in the filtrate is directly proportional to the concentration in the plasma I think that is the point that is pertinent here

Swann· Does the renal plasma flow change in these experiments?

Shock In the human kidney, plasma flow may fall from normal levels of around 600 ml per min to 250 ml per min when plasma hemoglobin levels are around 50-100 mg per 100 ml (19)

Dock With hemoglobin, immediately?

Shock Yes, with hemoglobin the decrease in flow can be detected within the first twenty minutes

Darrow Do any other proteins appear with increases in hemoglobin?

Dock Proteinuria does not increase during studies of hemoglobin excretion

Chinard We observed an increase in the glomerular clearance of albumin in patients with nephrotic syndrome who were given albumin Following the administration of albumin intravenously, there was an increase in the concentration of albumin in the plasma, but the clearance of albumin increased much more than the clearance of creatinine (which also increased) This may have something to do with pressure relationships, and it was suggested at one time that the effect might be related to stretching the membrane – if the membrane can be stretched

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POSSIBLE MECHANISMS OF FORMATION OF GLOMERULAR FLUID*

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THIS PRESENTATION WILL emphasize the mechanism of passage of substances across the glomerular capillary walls It is concerned with working hypotheses and experimental methods of attack rather than with definitive results

On the assumption that secretory activity is absent, there are two possible mechanisms by which water and other substances can cross capillary walls (*a*) filtration and (*b*) diffusion Definitions of these two mechanisms follow *Filtration* occurs because of hydraulic pressure gradients Proteins remain in plasma because of the sieve-like structure of the capillary walls, water and other substances pass in bulk through pores without separation of any of these smaller substances It is generally assumed that laws of hydraulic flow (including Poiseuille's law) are applicable to this flow through pores Diffusion effects are assumed to be negligible *Diffusion* occurs because of gradients of chemical potentials of the individual substances The rate of net passage is proportional to the diffusion coefficient of the substance in the vessel wall All substances, including proteins, cross the wall at a finite rate However, the diffusion coefficient of a small substance in the wall may be close to its diffusion coefficient in water, while the diffusion coefficient of a large substance, such as a protein, may be so small as to be negligible The factors which determine the gradients of the chemical potentials are concentration gradient of the substance considered concentration gradients of other substances, gradient of pressure, and gradient of temperature The gradient of temperature is considered to be zero across the glomerular capillary walls Diffusion can take place as the result of pressure gradients alone

Knowledge of the mechanism would permit deductions as to structure of the capillary wall If the mechanism is filtration, must visualize pores of specific dimensions in an otherwise permeable surface, movement through these pores can occur o

* This work has been supported by a grant from the Life Insurance Med Research Fund The experiments were carried out in collaboration with Dr L B Flexner

in the direction of the pressure gradient If diffusion is the mechanism, then one can visualize a latticework of fibers of molecular dimensions, through this latticework individual molecules or particles move in random fashion Across any given area of a wall, substances may move in both directions at high rates *Net* movements, the results of gradients of chemical potentials, take place at much slower rates

From thermodynamic considerations, it is possible to derive expressions for the glomerular clearance of any substance and for the rate of formation of glomerular fluid (GFR) without any assumption as to the mechanism of passage The basic assumption (cf de Groot(1) and Prigogine(2)) is made that the net rate of passage, $\frac{dS_J}{dt}$ of any substance, J, is proportional to the product of the chemical potential gradient of J, $\frac{\Delta\mu_J}{\Delta X}$, the area, A, across which passage takes place, and the concentration of J, [J], on that side of the wall where its chemical potential is greatest For small gradients we can write

$$\frac{dS_J}{dt} = K_J [J]'' (\mu_J'' - \mu_J') \frac{A}{\Delta X} \qquad [10]$$

The ″ refers to the blood side of the wall and the ′ to the glomerular fluid side K_J is a coefficient which depends on the mechanism and on the substance considered ΔX is the thickness of the capillary wall across which the gradient exists (It is assumed that passage from blood to glomerular fluid occurs along the whole length of the glomerular capillaries and that therefore $\mu'' > \mu'$) Differences of chemical potentials can be expressed as

$$\mu_J'' - \mu_J' = \overline{V}_J(P'' - P') + RT \ln \frac{N_J''f_J''}{N_J'f_J'} \qquad [11]$$

$\overline{V}_J$ is the partial molal volume of J, P is the hydraulic pressure, R is the gas constant, T the absolute temperature, ln denotes natural logarithm, N_J mole fraction of J, and f_J mole fraction activity coefficient of J By combining [10] and
[11] there is obtained

$$\frac{dS_J}{dt} = K_J [J]'' \frac{A}{\Delta X} \cdot \left[\overline{V}_J(P'' - P') + RT \ln \frac{N_J''f_J''}{N_J'f_J'}\right] \qquad [12]$$

On rearrangement, this becomes

$$\frac{1}{[J]''}\frac{dS_J}{dt} = K_J \frac{A}{\Delta X}\left[\overline{V}_J(P - P') + RT\ln\frac{N_J'f_J''}{N_J'f_J}\right] \qquad [13]$$

which is the glomerular clearance of J

For water instead of the term involving mole fractions, its equivalent, V_{H_2O} ($\pi - \pi$) can be written, $\pi - \pi'$ is the colloid osmotic pressure as conventionally estimated For water [13] becomes

$$\frac{1}{[H_2O]''}\frac{dS_{H_2O}}{dt} = K_{H_2O}\frac{A}{\Delta X}\overline{V}_{H_2O}\,(P - P - \pi'' + \pi') \qquad [14]$$

This is similar to the expression generally used for GFR, namely GFR − K (P′ − P′ − π″ + π) A strict expression for GFR from the thermodynamic formulations is

$$GFR = \sum_J \frac{dS_J}{dt}\overline{V}_J = \sum_J K_J\,[J]''\,\overline{V}_J\,\frac{A}{\Delta X}\left[\overline{V}_J(P'' - P) + RT\ln\frac{N_J'f_J'}{N_Jf_J}\right] \qquad [15]$$

The contribution of each constituent of the glomerular fluid to the volume of the glomerular fluid is summed for all the constituents This is a derivation of an expression for GFR It can be shown that [13] is applicable to a mechanism of diffusion or to one of bulk passage (If diffusion is the mechanism $K_J = \frac{D_J}{RT}$, where D_J is the diffusion coefficient of J in the wall In the case of hydraulic flow, on the assumption that Poiseuille's law applies, $K_{H_2O} = \frac{r^2}{\overline{V}_{H_2O}\,8\eta}$, where r is the pore radius and η is the viscosity)

Proteins cross the glomerular capillary walls in very small amounts in normal individuals and in relatively large amounts in patients with the nephrotic syndrome where the glomerular clearance of albumin is of the order of 0 5 to 4 per cent of the creatinine clearance A protein such as albumin does not "restrain" its own passage across the glomerular capillary wall and does not necessarily affect the passage of different substances to the same extent Expressions including osmotic pressure terms are not applicable to the passage of albumin and may not be strictly applicable to any substance but water It is preferable therefore, to use an expression such as [13] rather than one in which osmotic pressure terms appear

The effect of a sudden increase in protein concentration in plasma on the passage of water and of creatinine can be predicted from [13] without any assumption as to mechanism A sudden increase in the concentration of protein will not change the concentration of water relative to the concentration of creatinine ($[H_2O]''$ $[Cr]''$ and N''_{H_2O} N''_{Cr} will not be changed) However, f''_{H_2O} should be decreased much more than f''_{Cr} (Creatinine is normally present in plasma in such small molar concentrations that its solution in plasma may be considered ideal Water is approximately 53 molar in plasma It can be seen from the graph of Scatchard and his co workers(3) that at high protein concentrations the effect of changes of protein concentration on f''_{H_2O} is exponential rather than linear Changes in f''_{Cr} should be much less than changes in f''_{H_2O}) Therefore, following the rapid injection of protein into the renal artery, one would expect to find an increase in the concentration of creatinine in the urine relative to the concentration of water

This is put to experimental test as follows Anesthetized mongrel dogs are used A flank incision is made and the aorta and origin of the left renal artery are exposed by a retroperitoneal approach The injection is made through a needle introduced into the aorta and then turned up into the renal artery, the renal artery itself is

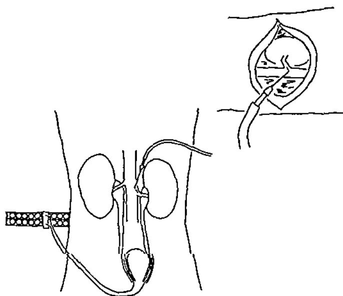


FIGURE 11 Operative approach

not touched (Figure 11) The ureters are catheterized urine is collected during periods of four to thirty seconds duration To obtain adequate urine flow, a mercurial diuretic or mannitol is given

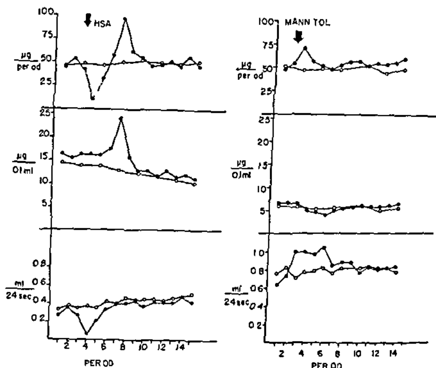


FIGURE 12 Effects of human serum albumin and of mannitol on water and creatinine excretion Ordinates top creatinine excretion in µg per period middle *creatinine concentration in µg per 0 1 ml bottom urine flow in ml per 24 second* period HSA 5 ml of 25 per cent human serum albumin injected Mannitol 1 ml of 15 per cent mannitol injected Black dots experimental kidney circles control kidney

Figure 12 illustrates the result of suddenly injecting 1 25 grams of human serum albumin in a volume of 5 ml (the injection took eleven seconds) into the renal artery No significant changes oc*curred in the control kidney In the experimental kidney, there was* an immediate decrease in urine flow with a delayed increase in the concentration of creatinine in the urine and in the excretion of creatinine per period (Each period was twenty four seconds) That the effect on urine flow is immediate is ascribed to the fact that the whole system from glomerular fluid to catheter tip is a connected hydraulic system The delay in the rise in the concentration of creatinine is ascribed to the time required for fluid to travel from

the glomerulus to the catheter tip The conclusion is that the thermodynamic formulation is compatible with the data

As a control, an equal volume of isotonic NaCl is injected There are very slight changes in the creatinine concentration and urine flow (Experiments in which sulfhydryl compounds are injected into the renal artery indicate that inhibition of a mercurial diuresis is not immediate but requires five to eight minutes to become fully established Similarly, diuresis by a mercurial takes five to eight minutes to be fully established)

Pappenheimer How do you know that the hydrostatic pressure of the capillary has not been changed as a result of the albumin?

Chinard If the pressure has been changed, it has probably been increased

Swann How do you know that spasm did not occur?

Chinard We don't The experiment is, admittedly, not conclusive The fact remains that the thermodynamic formulation is compatible with the experimental result A major objection not yet mentioned is that the effect of protein may be mediated at the level of the peritubular capillaries in the process of reabsorption of water This possibility cannot be eliminated In any case, the increase in protein concentration in renal arterial blood is followed by a decrease in the rate of excretion of water protein acts directly as an "antidiuretic," as would be expected from the formulations above, although not necessarily solely as a result of action at the level of the glomerulus but possibly also as a result of action at the level of the peritubular capillaries The effects of the injection of 1 ml of a 15 per cent solution of mannitol are also shown in Figure 12 The diuretic effect is evident

Under the usual conditions of clearance studies, changes in the concentration of the test substances are kept to a minimum To determine whether filtration or diffusion is the mechanism involved, it is necessary to arrange the experimental conditions so that the concentrations in plasma of the test substances are changed rapidly and proportionately If filtration is the mechanism, the clearance ratios of the test substances (assumed to enter the urine only at the level of the glomerulus) should remain unchanged in spite of rapid changes in the concentrations in plasma If diffusion is the mechanism the clearance of creatinine, which has a higher diffusion

lary wall In the first set of experiments, an "instantaneous" in

tion of a mixture of test substances was made into the renal artery, urine was sampled from each ureter for a number of periods of twelve seconds each (In an "instantaneous" injection there is first a very rapid rise in the concentration in the plasma, then a leveling off, and finally a rapid fall A solution may be injected in a "square wave" but the dynamics of flow are such that the material is rapidly spread out We have found no evidence for separation* of such substances as inulin and creatinine following injection into the aorta via the carotid and sampling from the femoral artery No evidence of separation was found after injection into the right auricle and sampling from the carotid) Figure 13 shows the results of an

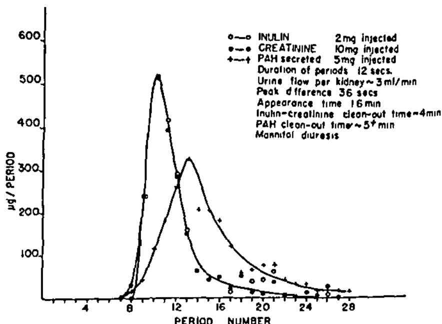


FIGURE 13 "Instantaneous" injection of mixture of inulin creatinine and PAH Ordinates excretion per period Abscissa period number PAH secreted calculated from PAH excreted minus inulin excreted

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* The significance of the separation of the peaks of the inulin and the PAH curves was not discussed because of the lack of time The separation is interpreted to mean that the rate limiting process in the secretion of PAH is either the transport across the tubular cells or the liberation from the cells into the tubular lumen

underestimates

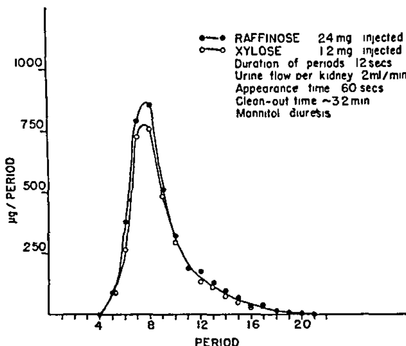


FIGURE 14 "Instantaneous" injection of mixture of xylose and raffinose

experiment in which inulin, creatinine, and PAH were injected Creatinine is present at the beginning in a higher concentration than inulin There is separation PAH is likewise present at the beginning in a higher concentration than the inulin

Figure 14 shows the result of an experiment with xylose and raffinose Xylose is reabsorbed more completely than raffinose The concentration of xylose is equal to that of raffinose at the beginning This is evidence for separation, if bulk passage occurred, the concentration of xylose should be less than that of raffinose Figure 15 shows the results of an experiment with inulin and ferrocyanide No significant separation was found The method for the determination of ferrocyanide are not sufficiently precise to permit any conclusion other than that the clearances are essentially the same (In all these experiments and most of those which follow it was possible to correct for recirculation by subtraction of the amount of the test substances excreted in the corresponding period from the control kidney All results are corrected for differences in the amount of each test substance injected The assumption is made that the

FIGURE 15 Instantaneous injection of mixture of ferrocyanide and inulin

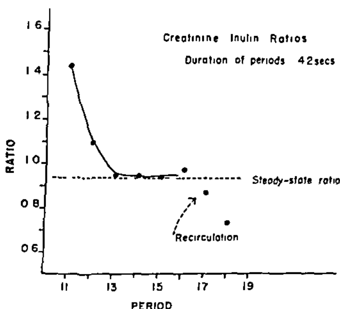


FIGURE 16 Logarithmic injection of mixture of creatinine and inulin Ordinate ratio of creatinine excreted to inulin excreted Results not corrected for recirculation Steady state ratio indicated by dotted line is clearance ratio for experimental kidney when the concentrations of creatinine and of inulin were kept constant

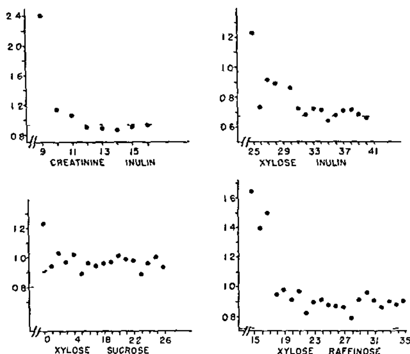


FIGURE 17 Logarithmic injection of mixtures of substan
rat
pc
pc
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effects observed occurred at the level of the glomerulus and not in the course of the passage of the urine along the tubules The linear velocity of the urine in the tubules must be of the order of several millimeters per second diffusion effects in the tubules are therefore considered to be negligible)

The instantaneous injection technique does not give sufficient information because the increase in concentration is not a sustained one Accordingly injection of the mixture of test substances in other experiments was made mechanically at a logarithmic rate The results are shown in Figures 16 and 17 for different test substances In all experiments with the possible exception of the xylose sucrose experiment significant differences were found in the excretion ratios in the early periods The differences are particularly large in the creatinine inulin and xylose raffinose experiments

INULIN 4mg injected
FERROCYANIDE 10mg injected
Duration of periods 12 secs
Urine flow per kidney ~175 ml/min
Appearance time 12 min
Clean-out time ~3+ min
Mannitol diuresis

FIGURE 15 Instantaneous injection of mixture of ferrocyanide and inulin

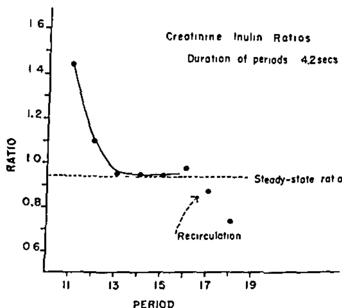


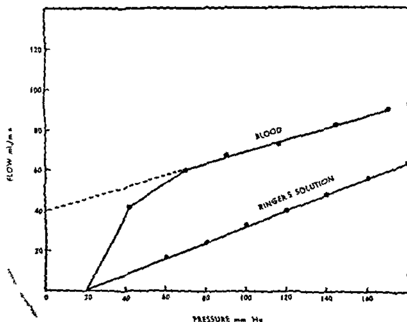
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·NTRARENAL PRESSURE AND
ΓNAL BLOOD FLOW

FRANK R WINTON

Department of Pharmacology
University College London England

·w goivu to talk about some rather simple properties of the dney and some ways in which I have tried to explore them In ·w of the previous discussion it seems more suitable to take up 'ood flow first and intrarenal pressure in a rather subsidiary way ter that I shall break off after discussing interaction between trarenal pressure and blood flow without developing other aspects intrarenal pressure very far Some others here take a very dif rent view of intrarenal pressure from mine, and I think that might a promising field for later discussion



8 The effects of arterial pressure on the flow of blood and of Ringer's the isolated perfused kidney of the dog at 37° C Values of flow shown of values measured during stepwise increases and stepwise decreases

Because the actual rate of increase of the concentration of the test substances in the plasma was small (the maximum rate of injection of the mixture is calculated to about 10 per cent of the renal blood flow), these must be considered preliminary experiments

These experiments are considered to lend support to the working hypotheses (*a*) that *substances cross capillary walls by a process* of diffusion rather than by a process of filtration, and (*b*) that the glomerular capillary walls, as well as the walls of the other capillary networks in the body, may be likened to gel-like structures made of fibers of molecular dimensions, rather than to impermeable plates with discrete holes of relatively uniform radius

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cumstances in which active physiological responses are absent? Or is it an affair which depends on the special properties of the blood, as a heterogeneous liquid, possibly in connection with the fact that a rather substantial fraction of water in the blood is withdrawn as glomerular filtrate leaving more viscid blood behind(4)

Let us consider the last possibility first One way of approaching the problem is to perfuse a kidney with Ringer's solution, because Ringer's solution can not be made more viscid by withdrawing filtrate in the glomerulus The difficulty with this approach is that the Ringer's pressure flow curve, though admittedly of the same general form as that of blood pressure flow curves of all other organs in that the flow *increases proportionately* more than the pressure, is otherwise quite unexpected (Figure 18) The Ringer flow is much less than the blood flow at the same perfusion pressure, although the viscosity of the Ringer's solution is also much less, and that shows that the whole mechanism has been completely disoriented When Ringer's solution reaches the kidney, the organ immediately expands and becomes very tense The main resistance to the perfusion of Ringer's solution in the kidney is not, then, the normal vascular resistance in the kidney at all, but an entirely abnormal development of intrarenal pressure compressing the vessels There may be other factors involved, but that certainly must be one major factor, and that particular approach to this problem is not, therefore much help in solving it

Selkurt Have you tried using plasma alone?

Winton Yes I have I got down to about 3 or 4 per cent corpuscles but I found it extremely difficult to keep kidneys in steady-states under those conditions A rather rapid drift sets in, so I haven't any reliable quantitative data about it

Selkurt I've tried a pectin solution which has the osmotic properties of plasma, then one gets a curve like your Ringer's showing a straight line relationship

Winton What was the oncotic pressure of that?

Selkurt The same as plasma It is a plasma substitute that was used during the war, and we did some investigation in our department to ascertain its properties I used it, incidentally to investigate the same problem you are investigating here, because it does reduce or minimize, the edema caused by Ringer's Nevertheless, we do get the straight line relationship with that solution, such as you found with Ringer's

Winton That is very interesting

Figure 19 indicates that this characteristic phenomenon still per-

The particular problem about blood flow in the kidney which I want to discuss is shown in Figure 18 There is general agreement about the facts qualitatively, but some divergence of views quantitatively Everyone, I believe, who has investigated blood flow in the kidney agrees that it changes much less in that organ with change in arterial pressure than it does in any other organ(1) This property of the kidney does not differ much, as far as we know, in the kidney of the intact animal or the denervated kidney in the anesthetized animal or the isolated perfused kidney I propose, therefore, mainly to consider isolated perfused dog kidneys because a direct method, i e stopwatch and measuring cylinder, can be used to measure the blood flow and one need not worry about whether an indirect method of measurement is right or wrong One can arrange manometers to be close to the artery, vein, ureter, and so on, and thus, the problem becomes simpler to think about than in the whole animal where one always has to keep an eye on the question of whether indirect methods are really measuring what they claim to be

The phenomenon everybody is agreed about, I think, is that the arterial pressure produces an increase of blood flow, some say next to none, others say much less than would be expected I hold that it is much less than one would expect In practice, this characteristic property only obtains above an arterial pressure of 50 or 60 mm of mercury Below that pressure, the blood pressure flow curve in the kidney is similar to that which is found in any other organ in the body that is to say, the blood flow increases more, proportionately, than the blood pressure (Figure 18) But above that pressure, the characteristic renal response is that the blood flow increases much less than proportionately with the blood pressure, usually following a fairly straight line until, at very high pressures, the blood flow begins to increase relatively more rapidly If you extrapolate the straight part of this line backwards, it generally intercepts the flow axis somewhere between 40 and 60 ml per min in a kidney of average size, 50 ml per min is about the average intercept in kidneys that I have had under observation These are usually kidneys taken from rather small dogs their weight ranging from 6 to 10 kilograms

The problem is how does this increased resistance to blood flow at increasing arterial pressure come about? Is there a physiological response of the kidney, for example, an automatic vasoconstriction, such as Rein(2) and Homer Smith(3) have suggested? Or is it a more or less mechanical affair which can be demonstrated in cir

cumstances in which active physiological responses are absent? Or is it an affair which depends on the special properties of the blood as a heterogeneous liquid possibly in connection with the fact that a rather substantial fraction of water in the blood is withdrawn as glomerular filtrate leaving more viscid blood behind(4)

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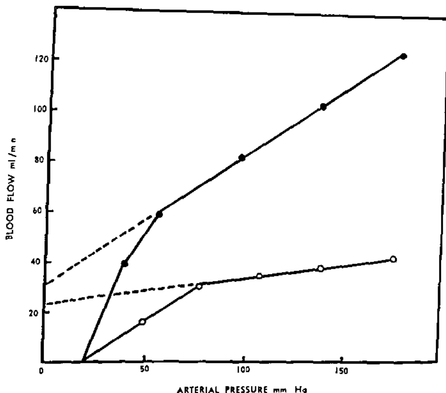


FIGURE 19 The effects of arterial pressure on blood flow in two isolated perfused kidneys (dog) at 4° C Values of flow are the means of values obtained during stepwise increases and decreases of pressure The two curves show the range of variation observed in different cooled kidneys

sists at very low temperatures The Figure shows blood pressure flow curves in two different kidneys at 4°, and the curves have the same unique form as in the kidney at body temperature The upper curve represents an unusually large kidney which happened to have a blood flow at 4° C roughly equal to the flow of the smaller kidneys already described at body temperature, both flows were taken at 120 mm Hg This makes it possible to compare the slopes of the cold and warm renal pressure flow curves by noting the values of the intercepts on the flow axis In cold kidneys, on an average, the resistance to blood flow increases a little less ... sure than in warm kidneys, but the ... phe... still well marked

The changes in resistanc... at d... al pres sures are summarized in F... h show... for a fairly large s... (a) ... ys

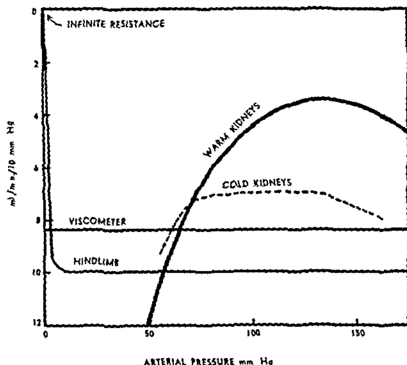


FIGURE 20 The effects of arterial pressure on mean resistances to blood flow of (*a*) kidneys at body temperature (*b*) kidneys from 3 to 12 C (*c*) hindlimbs at body temperature and (*d*) a glass viscometer 1 mm diam The calculation of resistances so as to make them comparable is explained in the text The curves show that at physiological arterial pressures the hindlimbs have a lower resistance and both sets of kidneys a higher resistance than that of the viscometer Cold kidney resistances are intermediate between those of warm kidneys and that of the viscometer

value of 100 ml per min at a pressure of 120 mm Hg and the reciprocal of the resistances to blood flow (in ml per min per 10 mm Hg) at all pressures have been multiplied by this same factor

It will be seen that in warm kidneys the resistance rises steeply at low arterial pressures until it reaches a broad maximum between approximately 90 and 160 mm Hg declining again at higher pressures In cold kidneys the maximum resistance is considerably lower than in the warm kidneys but the change in resistance with

pressure has the same general form In the glass viscometer the blood flow substantially follows Poiseuille s law and since it is scaled to 100 ml per min at 120 mm Hg the reciprocal of the resistance remains constant at all relevant pressures at the value 8 3 ml per min per 10 mm Hg As shown the resistance of the hindlimb is lower than that of the glass tube and this would be true also of all other organs such as the lungs coronary system etc with the single exception of the kidney where the resistance is higher than *in the glass tube when the arterial pressure exceeds 60 to 70 mm Hg*

The curious circumstance that this resistance phenomenon does not begin to appear in the kidney until arterial pressure exceeds 60 mm Hg or thereabouts may be coupled with another curious circumstance of the same kind The arteriovenous oxygen difference in the kidney also remains practically constant and independent of blood flow above that pressure As far as I know this property is unknown in other organs Again below that pressure the kidney resembles all other organs in showing increasing arteriovenous oxygen difference at lower blood flows There is a third property of the kidney which appears at just about the same arterial pres sure and that is the production of glomerular filtration and forma tion of urine above that pressure Those three unique properties taken together made me wonder whether it was not really glomeru lar filtration itself which might be responsible for the resistance phenomenon and if it could be explained on the basis of the in creased viscosity of the blood remaining in the vasa efferentia(4)

When I first became interested in these things no measurements had been made of the viscosity of blood in very small tubes Only measurements of the viscosity of blood more or less concentrated in relatively large glass tubes were available In order to obtain values of the viscosity appropriate to tubes the size of arterioles and capillaries we made a series of viscosity determinations in the hindlimb of a dog(5) which were intended as a prelude to carrying out similar observations on kidneys Unfortunately the experiments on kidneys gave us such varied results in a given kidney at different times that we did not feel they were fit to publish However the values we obtained in the hindlimb were used for calculating the amount of glomerular filtrate which would have to be withdrawn from blood to get this curious slope of the blood pressure flow curve Figure 21 shows how the calculation was made(1) It is only an approximate graphical method and more detailed mathematical analyses have been made by others since(3) but I think it tells all one really needs to know for this purpose

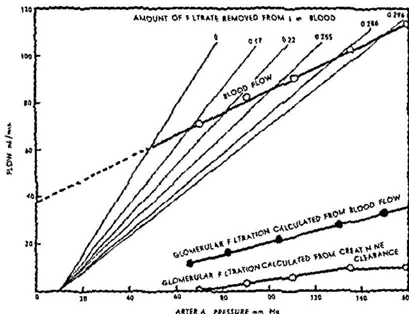


FIGURE 21 Observed effects of arterial pressure on blood flow and creatinine clearance of a perfused dog kidney at 37° C and the theoretical construction needed to deduce the influence of change in blood viscosity associated with the concentration in the glomerulus.

The assumption is first made that as far as the blood pressure flow curve is concerned the kidney behaves like a hindlimb below the arterial pressure at which glomerular filtration starts which I have taken to be 50 millimeters here The hypothetical blood pressure blood flow curve for the kidney can then be obtained in the absence of glomerular filtration by drawing a straight line joining the blood flow at 50 mm Hg to an intercept of 10 millimeters on the arterial pressure axis which from experience we found was approximately the curve characteristic of the hindlimb The line is not straight at very low pressures because it curves around towards the origin but for higher arterial pressures the hindlimb will give a quite straight line as in the Figure

A family of straight lines is then drawn fanning out from the intercept with slopes calculated from the increased viscosity of blood produced by concentration of the corpuscles and the increased plasma protein concentration when various proportions of ultra filtrate have been removed The points at which these lines intersect the observed renal pressure flow curve would on this hypothesis

give the glomerular filtration rates at the corresponding arterial pressures if the blood in the whole renal circulation had in fact become more concentrated

We imagine, however, that the part of the renal circulation traversed by the concentrated blood is confined to the region between the glomerulus and the peritubular capillaries where the water has been reabsorbed I shall take the pressure fall in this region as one half of the total arteriovenous pressure fall, although this amounts to little more than a guess, for the following reasons The glomerular capillary pressure may be taken as about two thirds of the arterial pressure(6,7) Taking values of peritubular capillary pressure as about the same as capillary pressure in other tissues and adding a little for intrarenal pressure, about one sixth the arteriovenous pressure fall is accounted for by the fall beyond the peritubular capillaries Clearly then, if the pressure fall along vessels traversed by the concentrated blood is only one half the total pressure fall from artery to vein, the increase in pressure needed to drive the concentrated blood through these vessels will also be one half of that calculated as though the concentrated blood flowed through the whole circulation

Figure 21 shows the observed creatinine clearance measured at the same arterial pressures as the blood flows, and it seems at first sight clear that the glomerular filtration rate is much too low to allow the supposition that increased viscosity of blood is the sole mechanism involved in this increased resistance to arterial pressure These experiments were however, done some time before the war and the technique of perfusion of kidneys in those days involved defibrinated blood and glass and rubber tubing The glomerular filtration rate in those kidneys was only, on the average about half what it had been in the kidneys before one removed them from the dog Nobody has a really satisfactory explanation for it It might even be due to reabsorption of creatinine The discrepancy between the observed creatinine clearances and those calculated as necessary to increase the viscosity of the blood cannot, therefore, be taken as conclusive evidence against the hypothesis It will be noted however, that the filtration fractions required by the hypothesis indicated on Figure 21 are higher than those generally reported in kidneys in intact animals, and this constitutes further and more substantial evidence against the hypothesis It is clear from these considerations that, whereas this increase of viscosity of the blood is one of the factors concerned in this affair, it is by no means the only factor There must be at least one other major

factor involved

This is perhaps, a little on the theoretical side and one would like to check by more direct experiment a conclusion of that kind I have already mentioned the approach to the problem involving elimination of liquids like blood, thus preventing increase in viscosity, by Ringer perfusion, and I should like to know what happens with other plasma substitutes, if there are such, which do not produce, as Ringer s solution does, so great a rise in intrarenal pressure as to invalidate evidence derived from the pressure flow relations But there is another way one might approach the problem One might aim at keeping the filtration fraction steady at different arterial pressures

The first and most obvious way is to abolish glomerular filtration altogether Glomerular filtration can be abolished by ligaturing the ureter But this does not provide satisfactory evidence because if the ureter is tied the pressure in the ureter rises on the kidney side of the obstruction to the maximal ureter pressure and that rises with arterial pressure Unfortunately the ureter pressure itself has some effect on the blood flow It has much less effect than the venous pressure but a large increase in ureter pressure does diminish the blood flow If, therefore in a kidney with obstructed ureter an increased resistance to blood flow with increased arterial pressure is found it may be argued that this is only due to a rise in ureter pressure itself producing increased obstruction to the outflow of blood

Therefore I modified the experiments somewhat by trying the effect of varying glomerular filtration rate in two ways first by ureter pressure increases which keep the glomerular filtration rate constant in spite of increase in arterial pressure This procedure had relatively little effect on the slope of blood pressure blood flow curve Second the glomerular filtration rate can be greatly reduced by cooling kidneys within the physiological range of arterial pressure, as compared with that in warm kidneys (8) It is higher at low arterial pressures as shown in Figure 22, and in cold kidneys there is a urine flow at pressures too low to produce it in warm kidneys, just as if osmotic diuretics are used But over the greater part of the pressure range, the clearance in a cold kidney is markedly less than that of the creatinine clearance in a warm kidney and, in most experiments, it is reduced to about one fifth

The increase in resistance to blood flow with increasing pressure is a little less in cold than in warm kidneys (Figure 19), and I think that difference may be attributed in part, perhaps, to the lower

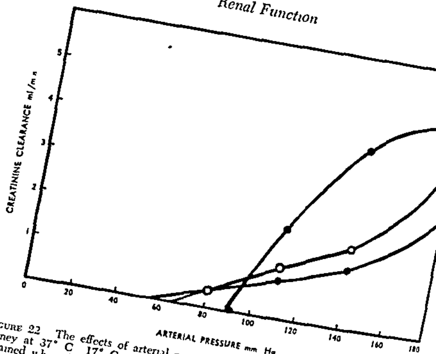


FIGURE 22 The effects of arterial pressure on creatinine clearances in a perfused kidney at 37° C 17° C and 3° C Each value represents the mean of values obtained when the pressure was raised and when it was lowered

glomerular filtration rate in the cold But, again, although the qualitative evidence supports the view of the analysis I have given (Figure 21), it does not in fact get one very much further, except to the point at which it is clear that the viscosity phenomenon is not sufficient to account completely for this increased resistance Perhaps some day the viscosity analysis in connection with clear ance can be done more quantitatively on kidneys with normal creatinine clearances thus enabling a more accurate estimate of what part it plays, but at the moment I think it can only be said that it plays a substantial, but probably not a dominant role, in the phenomenon

Let us consider, now, the hypothesis that automatic vasoconstriction accompanies increase in arterial pressure I do not, myself believe that physiological vasoconstriction can play any part whatever in this phenomenon This hypothesis seems to me to ignore the fact that if poisons are used to eliminate vasoconstriction the increased resistance to blood flow with pressure is, substantially speaking, unchanged The blood flow can be doubled with cyanide or halved by cooling a kidney to, say, 3°C and the phenomenon remains I have examined the effects of chloral hydrate in a series

of concentrations Chloral hydrate in concentrations exceeding about 0 1 per cent in my experience abolishes automatic activity in plain muscles of this kind I have perfused kidneys with concentrations up to 1 per cent and this phenomenon persisted It seems clear therefore that vasoconstriction in any physiological sense is not involved If there is any kind of change in resistance it must be some anatomical mechanical affair rather than an active physiological vasoconstriction

I am not I am afraid going to be able to give the solution to this problem We do not yet know it But before dealing with the controversial topic of intrarenal pressure I should like to call attention to the fact that perhaps the mechanism might be localized to some extent by tapping the circulation for evidence at the glomerulus By taking the creatinine clearance as indicating the glomerular filtration rate at least under conditions when creatinine and inulin clearances are the same(9) it can be determined whether this increased resistance to arterial pressure has already appeared at the glomerulus i e whether it is a phenomenon of the preglomerular part of the circulation or whether it has not yet appeared at the glomerulus in which case it is postglomerular The shape of this curve in Figure 22 which is perfectly characteristic of creatinine clearance in the warm kidney shows that the resistance is increasing with arterial pressure in the same general way as with the blood flow

The curves in Figure 23 are taken from a series of seventeen kidneys with the creatinine clearance resistance increasing to a maximum and then staying about the same or possibly declining a little The blood flow resistance increases to a maximum and then perhaps declines a little The creatinine clearance scale is one fifth of that of the blood flow scale but otherwise the curves are fairly alike

Pappenheimer Does that mean that if there were no change in the afferent arterioles as the pressure changed you would expect the straight line between them?

Winton If there were no change in afferent or efferent vessels let s say broadly pre or post glomerular circulations you would expect that the glomerular pressure would go up proportionately with the arterial pressure and there would be no change in resistance

Pappenheimer So this would be a straight line?

Winton Yes the resistances of both blood flow and glomerular filtration should remain the same

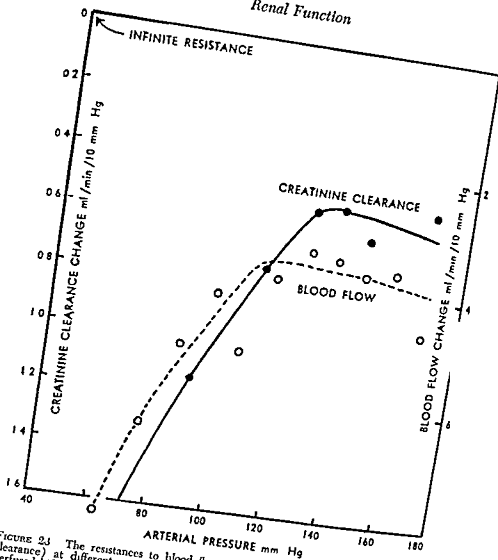
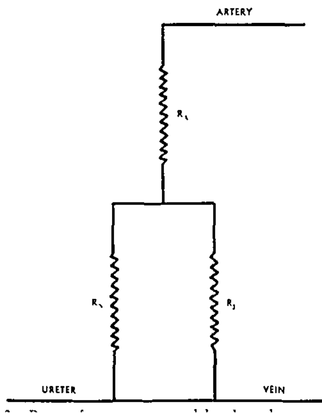


FIGURE 23 The resistances to blood flow and to glomerular filtration (creatinine clearance) at different arterial pressures Values are means from a series of 17 perfused kidneys at about 37° C The curves show general parallelism in the effects of arterial pressure on the two types of resistance

Pappenheimer But how would you form conclusions as to where the change of resistance is occurring?

Winton I am suggesting that the blood pressure flow re in the kidney may, in their simplest form, be represented model consisting of three elements (Figure 24) From the a single resistance (R_A) representing the preglomerular circu leads to two resistances in parallel, R_E representing the po

merular blood circulation and R_N representing the nephron At their lower ends, R_E and R_N connect with the vein and ureter, which are assumed to be at the same constant pressure and can be joined in the model If the increased resistance to blood flow with increased arterial pressure were located in the postglomerular circulation (R_F), this would produce a rise in glomerular pressure more than proportional to the rise in arterial pressure and, consequently, a fall in apparent resistance to glomerular filtration Since, on the contrary, experiment indicates a rise in resistance to glomerular filtration with increase in arterial pressure, I am suggesting that both this rise and the rise in resistance to blood flow are likely to



brane and tubules Vein and ureter are indicated at atmospheric pressure and therefore linked in the diagram

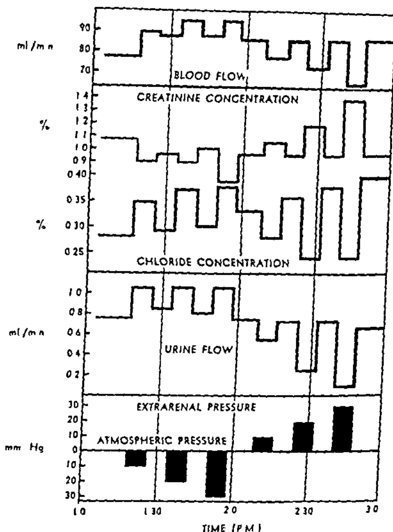


FIGURE 25 The effects of varying extrarenal pressure when arterial venous and ureter pressures remain constant on urine and blood flows and urine composition in a perfused kidney (dog) Urine flow is increased maximally by the smallest decrease in extrarenal pressure shown blood flow also being increased

sufficient to overcome the obstruction due to intrarenal pressure completely and further lowering could therefore have no effect In the later period of the experiment the effects of increasing the extrarenal pressure above atmospheric pressure are shown to be much the same as the effects of increasing the venous pressure

Experiments of this general kind provide fairly definite proof that

intrarenal pressure obstructs the outflow of urine, but the point I want to discuss particularly is the effect of intrarenal pressure on the blood flow Intrarenal pressure clearly exerts some obstruction to the blood flow, as indicated in Figure 25, though this does not necessarily imply that it obstructs the blood flow to the full amount, by which I mean as much as a corresponding increase of venous pressure would

There is another way in which the effects of intrarenal pressure can be examined by measuring the tenseness of the kidney If a sclerometer is placed on the surface of a kidney, that is to say, a disc of about 5 mm diameter, and the pressure necessary to depress

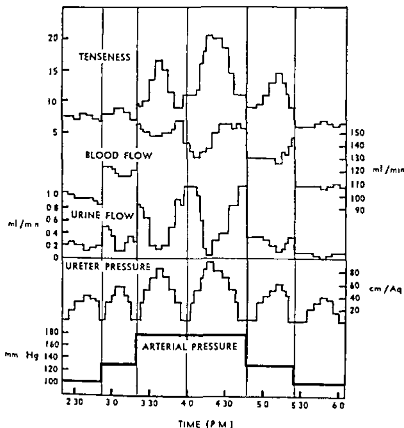


FIGURE 26 Charts showing the design of an experiment to discover the effects of ureter pressure on urine flow (intrarenal pressure) on blood flow and on the tenseness of the isolated dog kidney at three arterial pressures

it a small distance is measured, a qualitative estimate of the pressure in the renal substance can be obtained Figure 26 shows the changes of tenseness when arterial pressure and ureter pressure are changed, and incidentally shows the form an experiment takes when the effect of arterial pressure on ureter pressure, urine flow curves is being investigated Here are three arterial pressures, going up and then coming down again, so as to get the average of values obtained during rise and fall of arterial pressure These are all steady state values, the average values being necessary only to eliminate the effects of drift in time of the properties of the kidney since the experiment involves observations over a period of two and a half to three hours Stepwise increases in ureter pressure produce corresponding declines in urine flow At high arterial pressure the ureter pressure must be raised quite a lot, 70 mm Hg or so, to obtain the required decline in urine flow The effect of the arterial pressure on the blood flow is quite considerable, of course, but the effect of the ureter pressure on the blood flow is not very great until these quite high values are reached The tenseness of the kidney measured by sclerometer increases a little with arterial pressure and increases quite a lot more with ureter pressure But the important point is that the increase with arterial pressure is relatively small

Swann What units of tenseness are used?

Winton They are millimeters of mercury pressure required for a standard small depression of the sclerometer disc

Figure 27 shows the effects of osmotic (urea) diuresis and changes in arterial pressure on the reciprocal of the tenseness (compliance) of a kidney
tive circulat
lood in the
arliest observations, gives values referred to perfusion with added rea curve II corresponds with perfusion without added urea, and urve III gives values obtained after return to the urea perfusion ircuit The values plotted are compliances, measured in μ per mm [g, which means the higher the value, the more easily is the kidney urface depressed by the sclerometer disc

It is clear that the changes in compliances produced by changes 1 arterial pressure are rather uncertain and relatively small compared with the effects, say, of urea The no urea kidney is a kidney hich is much less tense than the kidney under the influence of rea, but above the lowest series of arterial pressures, where the enseness seems to fall off with pressure, there is little effect of

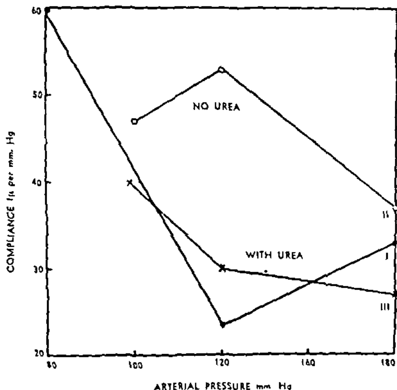


Figure 27 The effect of [illegible] a diuresis on the reciprocal [illegible] ed by the distance through [illegible] disc placed on the surface [illegible] in which the observations

change in arterial pressure on the tenseness of the kidney, and such small effects as there are are sometimes in one direction and sometimes in another

The tenseness of the kidney and the intrarenal pressure, as I measure it, seem therefore to correspond reasonably closely in their reactions to pressure and osmotic diuresis I have an impression that the tenseness varies rather more with arterial pressure than intra renal pressure, as shown in Figure 28

Figure 28 is a set of venous pressure, urine flow curves obtained in an experiment designed in the same way as indicated in Figure 26 Rises in venous pressure to values lower than the intrarenal pressure are apt to raise the urine flow due to the fact, I believe,

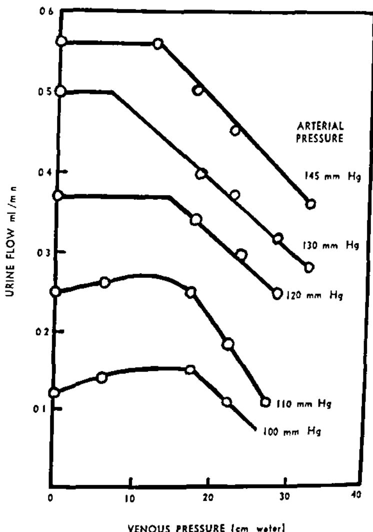


FIGURE 28 The effects of arterial pressure diuresis on intrarenal pressure as indicated by the lowest venous pressure capable of reducing urine flow (Dog pump lung kidney preparation)

that a rise in venous pressure is more effective than an equal intra renal pressure in obstructing the outflow of blood If the venous pressure is raised the glomerular filtration pressure of blood in the capillaries is raised but additional back pressure is not produced by compressing the tubules until the venous pressure exceeds the intrarenal pressure(17,7)

Experiments of this kind whether involving increase in venous

pressure or increase in ureter pressure do not yield any evidence that the intrarenal pressure is increasing with arterial pressure If, however, a family of curves of the same kind is examined but the urine flow is varied in each curve not by changing arterial pressure but by using different concentrations of an osmotic diuretic, it is found that the intrarenal pressure *is* much higher, from 30 to 40 mm Hg at high urine flows than at low flows My kind of intra renal pressure, as contrasted with Dr Swann's does not, therefore appear to be able to contribute anything to the explanation of the increased resistance to blood flow at higher arterial pressures

Berliner Before you go on to something else I wonder if you would repeat what you said about osmotic diuresis in this situation

Winton Osmotic diuresis, such as sulfate diuresis, produces a set of ureter pressure urine flow curves as shown in Figure 29 The intrarenal pressure is fairly low at a low urine flow and becomes higher the higher the diuresis It is quite unlike the effect of varying

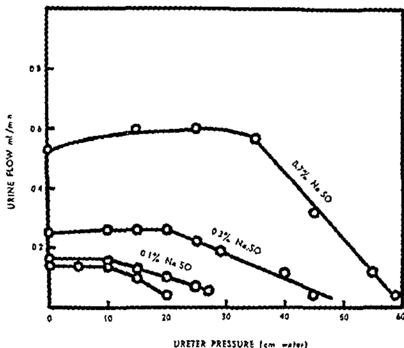


FIGURE 29 The effects of the degree of osmotic diuresis (sodium sulphate) on intrarenal pressure as indicated by the lowest ureter pressure capable of reducing urine flow (Dog pump lung kidney preparation B P 110 mm Hg)

arterial pressure (Figure 28) where there is high urine flow, but the decline in urine flow begins at the same ureter pressure as it does at low urine flows

Pappenheimer How high can it go under those conditions?

Winton The intrarenal pressure? I don't think I have measured anything over approximately 50 millimeters, but that involves pretty high diuresis However, I would not be at all surprised if, in Ringer diuresis, it were higher than that I have never been able to measure it in Ringer diuresis because urine flow falls off rather rapidly and there are not constant enough base lines from which to obtain ureter pressure urine flow curves

Last, I want to say a word about the relation between ureter pressure and blood flow Figure 30 shows how an increase in ureter

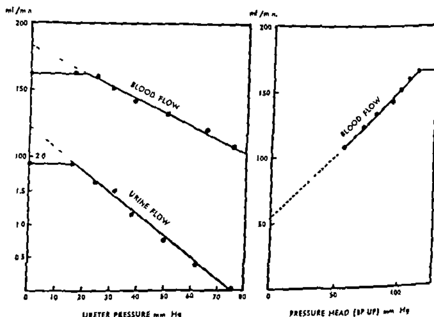


Figure 30 Left The effect of ureter pressure on the urine and blood flows of an isolated kidney at 36° C and at constant arterial pressure 130 mm Hg Right The relation between blood flow and pressure head (arterial minus ureter pressure) calculated from the blood flow curve on the left

pressure affects both the urine flow and the blood flow An increase has no effect until it exceeds the intrarenal pressure, and beyond this there is a reduction in both flows If blood flow is plotted against the pressure head, i e , blood pressure minus ureter pressure, there is quite a different result than is obtained from plotting pres

sure head varied by increasing the venous pressure In the latter case, the curve is approximately a straight line passing through the origin, in other words, the effect of raising venous pressure on the blood flow is to produce a reduction of blood flow proportional to the reduction of pressure head (apart from an uncertainty due to the change in the intrarenal pressure at the beginning) But ureter pressure, curiously enough corresponds much more closely with arterial pressure in this respect If the pressure head is reduced by increasing the ureter pressure, a blood pressure flow curve is found which has an intercept at zero pressure somewhere around 50 ml per minute just as is found if the pressure head is reduced by lowering the arterial pressure I do not know whether that has any significance at all beyond coincidence It may only be, as I have imagined, that when the tubules are distended by raising the ureter pressure, they will not expand beyond a certain point They are like elastic tubes surrounded by cotton tubes Beyond a certain point, differing for different tubules, they cannot expand, and there fore the blood vessels do not get so effectively compressed by dis tending the tubules as the tubules are by distending the veins

Bradley Does that happen in poisoned kidneys, too?

Winton I think it does I do not really have good measurements in poisoned kidneys because it is much more difficult to get good base lines I have examined these relations only after cyanide and chloral hydrate

There is a possibility, I think, that intrarenal pressure is having the same kind of effect due to tissue rigidity Intrarenal pressure, ureter pressure or arterial pressure produces only about half the change or less than half the change of blood flow per unit change in pressure that might be expected, for example, in a glass tube, whereas increase in venous pressure appears to produce the full reduction in blood flow that would be found in a glass tube

The question as to whether the interstitial pressure measured by Dr Gottschalk and Dr Swann by plunging needles into the kidney measures intrarenal pressure or something different is one which we will have to try to sort out If we come to the conclusion that Dr Swann's tissue pressure of 25 millimeters and mine of about 3 millimeters his being interstitial pressure and mine intrarenal pres sure both on the same preparations in so far as a Texas dog can be said to be the same as a London dog is to be explained by the fact that the tissue pressure is not transmitted fully to the lumen of the tubules, then one is up against a rather awkward phenomenon If the tissue pressure is increased by increasing the venous pressure,

say, to 30 millimeters of mercury, and the ureter pressure in the same kidney is now increased gradually, it has no effect until it reaches 30 millimeters of mercury (17) Beyond this pressure it begins to reduce the urine flow That looks as if the tissue pressure as raised by the venous pressure to 30 millimeters of mercury—and I believe Dr Swann agrees with me that they correspond—produces an obstruction of the tubules because, as indicated by this technique, the obstruction then becomes manifest at just about that same pressure At that point, I think I shall leave it

Swann We have been using a modification of Landerer's method for measuring tissue pressure(18) * We employ a cannula with lateral holes, it is actually a 20 gauge needle with the end plugged and 5 lateral holes bored along the shaft This cannula is inserted into the renal parenchyma Its distal end is connected with the apparatus shown in Figure 31 Pressure H is transmitted through stopcock S to the isometric manometer B B is actually a glass Bourdon tube optical registration of its deflections is made(19)

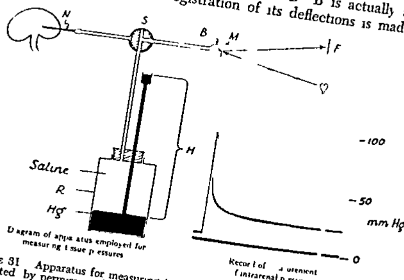


FIGURE 31 Apparatus for measuring tissue pressures and record of a measurement Reprinted by permission from Swann H G *et al J Exper Med 92, 626 (1950)*

When the stopcock is set in the position shown in Figure 31, the pressure H stretches the Bourdon tube B slightly, in actual volume about 4 to 10 cubic millimeters of fluid When the stopcock is rotated 90° counterclockwise, the pressure discharges into the renal

* The work reported here has been supported in part by the M D Anderson Foundation Houston Texas It has been conducted primarily by Messrs J C Davis B W Hink H Koester A V Montgomery V Moore and J M Prine

parenchyma, the Bourdon tube pushes a small volume of fluid into the kidney An illustration of the records obtained is shown in Figure 31 In this case, the Bourdon tube was stretched with a pressure of 90 mm Hg When it was allowed to discharge into the kidney, its pressure fell rapidly at first then slowly, and it finally came to rest at a pressure of 23 mm Hg This pressure we call the intrarenal pressure, or, accepting Dr Winton's suggestion, the interstitial pressure of the kidney

Many modifications of Landerer's old method have been used which depend upon observing the pressure at which a small volume of fluid is forced into a tissue But as McMaster has shown(20), when fluid is forced into a tissue its micro structure is distorted to a certain extent, therefore, in one sense a measure of the pressures required for distortion is what is obtained Hence the older techniques usually give falsely high values for tissue pressure But with the present technique, the pressure is measured at a no-flow point The pressure falls asymptotically until no fluid is moving into the tissue Thus, I think that we are not measuring distortion pressures

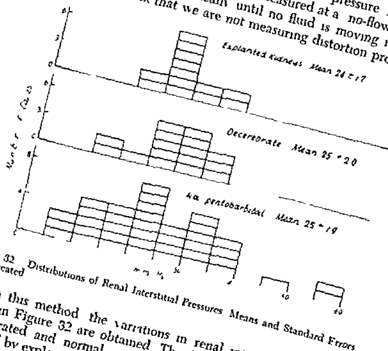


FIGURE 32 Distributions of Renal Interstitial Pressures Means and Standard Errors are indicated

With this method the variations in renal interstitial pressures shown in Figure 32 are obtained The data are for nembutalized decerebrated and normal unanesthetized dogs The latter were prepared by explanting the kidney to a subcutaneous position thus

after recovery the pressure may readily be taken by thrusting the needle through the skin and into the kidney The average in all groups is about 25 mm Hg In a group of dogs the standard deviation is about 11 mm Hg the range of variation is from 10 to 58 mm (14)

Fremont Smith Isn t it true that the assumption that you have come down to the normal intrarenal pressure at the asymptote is based on the belief that the extra fluid introduced into the semi rigid kidney has an ample opportunity to escape?

Swann Yes it has to drain away somewhere

Fremont Smith If it hadn t had a chance to drain away you would still be somewhat above the true intrarenal pressure

Swann Exactly The record would then show that the pressure was still falling very slowly We take no measurements until the pressure in the transducer is not changing

Pitts Suppose you repeated a determination immediately after having done one how does the second compare with the first?

Swann The second measurement checks well(19) If we repeatedly measure the pressure over a period of a couple of hours the standard deviation of multiple readings in a single animal is 1 2 to 3 mm Hg We can readily repeat the measurement over long periods of time provided we do not change the animal s physiological condition

Fremont Smith Have you ever had one needle in and then with a second apparatus put in another and taken the pressure to see what effect the second pressure had on the first?

Swann Yes the two check very nicely The needle can also be reinserted a number of times and the same reading obtained(19)

When we ascertain what happens in these kidneys after inserting the needle we find by histologic examination that a very considerable laceration has been made as would be expected by the 20 gauge needle(19) Many tubules are torn and forced through the tissue by pushing this big crowbar as it were through the tissue Many small blood vessels are torn and ruptured We therefore think that we are making an artificial pool into which the small volume of fluid from the manometer can be forced This fluid has to drain away through the many effluents available blood vessels lymphatics or tubules In the dead dog incidentally the intrarenal pressure is about 10 mm Hg it is constant and does not vary at all

We have many types of confirmation that the renal interstitial pressure is so high As you are all aware it is a very high tissue pressure It is the same as that in a maximally tensed gastrocnemius

muscle and the same as that of intraocular fluid

Fremont-Smith It is not the pressure though of cerebiospinal fluid

Swann No, it is not That pressure is around 10 mm Hg

Pappenheimer How much does it depend on the size of the needle?

Swann It is the same with a 27 gauge needle as with a 20 gauge needle

Pappenheimer And with a micropipette?

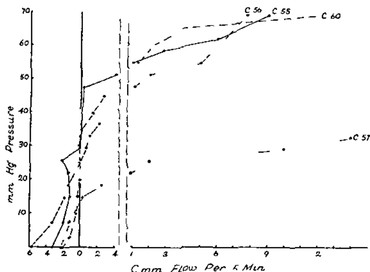


FIGURE 33 Relation of pressures to fluid flows out of (negative values) or into (positive values) a cannula set in the renal parenchyma Reprinted by permission from Montgomery A V *et al* *Texas Rep Biol & Med* **8**, 266 (1950)

Swann Figure 33 shows an experiment in which we used a micro pipette and microvolumes of fluid(11) We measured the pressure needed to force very small volumes of saline into the kidney This is the technique that McMaster has used so successfully in measuring intradermal and other tissue pressures(20) It is also the method that Gottschalk used to measure intrarenal pressure(13) We used a 27 gauge needle with lateral holes

Figure 33 shows this result if a pressure of less than 10 to 15 mm Hg was imposed on the cannula fluid flowed out of the cannula At these low pressures in other words the kidney forced fluid out of the cannula at rates shown on the abscissa of the graph As the

pressure was increased, a point of "zero" flow was reached—fluid moved neither into nor out of the kidney. At higher pressures still flow was inwards. The "zero flow" points varied from 13 to 44 mm Hg, the average was 26 mm for nine dogs.

This is confirmation from an independent type of experiment of the interstitial pressure observed with the first technique. Another confirmation is this(19): the apparatus first described was used. The needle was inserted into the kidney and we waited to see what would happen. The pressure very slowly rose and finally stopped at about 25 mm. The kidney forced fluid into the Bourdon tube until a pressure of 25 mm was reached. Here, then, are three different types of experimental techniques which give the same result. We feel, therefore, that our original measurement is a good one.

Still another confirmation is this: we reasoned that if there were a high tissue pressure in the kidney, the veins in the renal parenchyma would be collapsed unless their pressure was greater than the interstitial pressure(14,1). For that reason, we decided to attempt to measure the venous pressure by running a catheter all the way up into the veins of the parenchyma itself. The pressure here was to be ascertained. I shall describe the technique briefly (21): we use a small nylon catheter, connected with a nearly isometric manometer (actually a Bourdon tube with optic registration). The catheter is pushed up the renal vein blindly until it will not go any further. Then if it is withdrawn slightly, the pressure in it is about 25 mm Hg. If we take a simultaneous measurement of the interstitial pressure, using the needle technique first described, we find that the two measurements agree quite well: their coefficient of correlation, over a wide range of experimental values, is 0.85. I think that the reason why it is not better is that the measurement using the transduced equilibrium method is not giving nearly as accurate a measurement as is the venous pressure technique.

When we come to ascertain the position of the catheter's tip at autopsy, we find that it lies just above the confluence of the arcuate veins. The catheter has gone all the way up the interlobar veins and just barely turned the corner into the arcuate veins. The arcuate venous pressure, then, is about 25 mm Hg.

You will recall that the pressure in the main renal vein is about 11 mm Hg. And yet I have just reported pressures in the smaller renal veins of 25 mm. Where does the pressure change occur? Figure 34 shows an experiment in which the catheter was inserted into an arcuate vein, its pressure was 32 mm Hg. Simultaneous interstitial pressure, as measured with the needle, is shown to be

28 mm Hg, a fairly good correspondence between the two Now if we start pulling out the catheter slowly, we find the pressure changes along the course of the vein At the arrows in Figure 34,

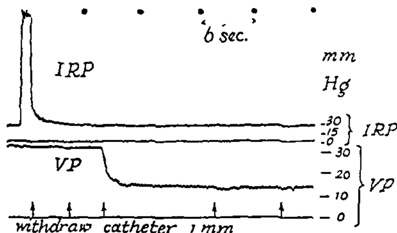


FIGURE 34 Venous pressure (VP) changes during withdrawal of renal vein catheter from arcuate into interlobar vein A simultaneous measurement of renal interstitial pressure (IRP) is shown Printed by permission from Swann H G, *et al.* The intrarenal venous pressure *Science* (In press)

we pulled out the catheter a millimeter at a time At the first two pulls, nothing happened Then suddenly, at the third pull, the pressure dropped from 32 to 14 mm the latter being close to main renal vein pressure We think, therefore, that the abrupt pressure drop in the renal venous system takes place near or at the arcuate interlobar junction

Fremont Smith To what extent do you think the catheter raised the pressure locally by obstructing the outflow in the arcuate vein?

Swann If it obstructed the *only* venous effluent, the pressure would rise to arterial But the veins of the kidney form very generous *anastomoses* with each other(22) A single region is never drained by just one vein All the arcuates 'arch over' into each other very freely Hence, we feel quite sure that although the catheter may block one arcuate vein the pressure does not increase locally be cause the blood in the blocked arcuate can so readily drain through neighboring arcuates

I wish to describe two other experiments which furnish indirect confirmation of our hypothesis that renal interstitial pressure is about 25 mm Hg The experiments just discussed have been fairly

direct measurements of renal interstitial pressure But there is also a lot of indirect evidence that this pressure is high One experiment which we did was as follows(23) A catheter was inserted in the renal vein Then the renal vein pressure was increased by partially occluding the vein with a tourniquet placed proximal to the catheter tip Simultaneously, the renal interstitial pressure was measured Figure 35 shows the results The interstitial pressure did not start

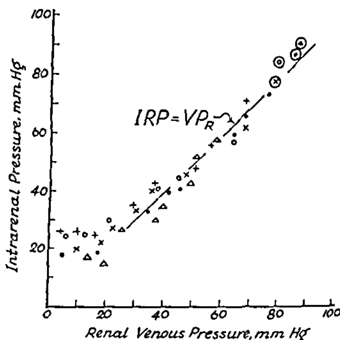


FIGURE 35 Relation of intrarenal pressure and renal venous pressure as the renal vein is progressively occluded Individual records of 5 dogs Encircled values were taken after complete occlusion Reprinted by permission from Swann H G *et al* *Proc Soc Exper Biol & Med* 76, 775 (1951)

to increase until the simultaneous venous pressure exceeded the control interstitial pressure When the venous pressure rose above 25 mm Hg an effect on interstitial pressure first became apparent After the venous pressure exceeded the control interstitial pressure, the two rose together, with further venous occlusion in a one to one relationship

Dr Winton has done the same sort of experiment One should not influence the interstitial pressure by raising the venous pressure until the venous pressure exceeds the interstitial pressure Exactly

this happened in the present experiment It seems to me that this is very good indirect evidence that the interstitial pressure is at the high level of 25 mm Hg

Another piece of evidence in favor of the hypothesis is to be found in the experiments measuring the effect of venous pressure on renal blood flow Dr Blake and his group(24) and Dr Selkurt and his(25) have both measured the effects of partial occlusion of the renal vein on renal blood flow In both experiments, either minimal or no effect on blood flow was caused by increasing the venous pressure to about 20 mm Hg It was only when the venous pressure was raised far above 20 mm that the blood flow was slowed The interpretation is obvious because the renal interstitial pressure is 25 mm Hg, flow of blood through the organ is not influenced until the vein pressure exceeds interstitial pressure

Berliner You referred to the data of Dr Blake and Dr Selkurt(24,25) Although they could find no change in blood flow at lower venous pressures, they did find changes in water and electrolyte excretion How would you interpret that?

Swann According to my theory, raising the venous pressure should not influence any of the kidney s functions, including the rate of blood flow, until the venous pressure exceeds renal interstitial pressure

Selkurt I might add, in connection with that, that we did get small changes in blood flow and small reductions in filtration rate at the lower venous pressures, but the change in sodium and water excretion came on more markedly and dramatically above 20 millimeters of mercury

Fremont-Smith Do you want to make any further comment on the dramatic change in intrarenal venous pressure between the arcuate vein and the interlobar vein?

Swann In order to speak about that, I shall have to make a digression into ocular physiology In the eye, there is also a very high interstitial pressure, for the pressure in the aqueous humor is 25 mm Hg This is the same as in the kidney In fact, the eye and the kidney have extraordinary similarities in structure, and they also experience somewhat the same manifestations of disease Striking parallels may be drawn between their physiology and their anatomy * We got our first hints as to what sort of structure we might find in the arcuate interlobar region from examination of the

* Examples the ciliary process resembles closely the glomerular tuft in both organs a nearly protein free filtrate" is formed in hypertension or eclampsia both are subject to great structural changes arterial occlusions of varying degrees may occur in both with similar hemodynamic and pathological results

ocular veins and, specifically, the vortex veins

Reviewing briefly the venous circulation of the eye, the main venous drainage is through the vortex veins The venules of the uvea form a sinus where their tortuous, radial confluences run together Out of this springs a vortex vein (there are usually four of them) to run obliquely through the sclera and emerge just behind the equator of the eyeball As you would expect, with a pressure inside the eyeball of 25 mm Hg, the intraocular venous pressure is higher than 25 mm Hg as Duke-Elder has shown(26) It has to be or else the vein would collapse Just at the sinus, the pressure is about 2 mm higher than intraocular pressure while just at the exit of the vein from the sclera the pressure falls to 18 and then a little further on to the general venous pressure of the head The sharp change in pressure along the vortex vein is similar to the change observed in the arcuate-interlobar region of the kidney veins

The only detailed study of the vortex vein that I have been able to find is that of Fuchs in 1884(27) This, I think shows exactly what is going on In an illustration in Fuch's report, a vortex vein is shown running diagonally through the sclera It is a large vein, carrying much blood, but its walls are very thin In the middle of the sclera it is only about 0 004 mm thick Then suddenly, just at the exit from the sclera, its walls thicken to 0 04 mm, or about ten-fold

A cross section is shown in the lower part of Fuch's illustration The thin walled vein is mostly surrounded by a lymphatic A knot of connective tissue lies buried in the sclera It eventually runs to the side of the vein We make the guess that the connective tissue anchors the vortex vein inside its lymphatic channel Fuchs calls them side fastenings"

Let me go back for a moment to explain why these structures are so interesting Because we wished to study fluid flows through elastic vessels surrounded with a high interstitial pressure, we constructed the model shown in Figure 36(23) A reservoir with the pressure head H forces water through the system The water flows through elastic tube E, actually Penrose tubing Tube E is surrounded by rigid chamber K, which is filled with water and which has imposed upon it the pressure head T When fluid is flowing through this model pressure T opposes pressure H Therefore pressure T should reduce outflow from the system by one method or another It does it by increasing the resistance, what happens is that a constriction forms between A and B The greater pressure T is, the more marked the constriction Because the constriction is

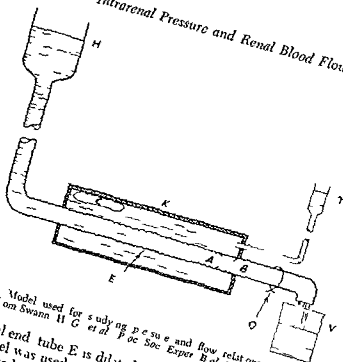


FIGURE 36 Model used for studying pressure and flow relations Reprinted by permission from Swann H G et al Proc Soc Exper Biol & Med 76 75 (1951)

at the distal end tube E is dilated proximally

This model was used to study flows through elastic tubes which were subjected to outside pressure We never thought originally that we would find any anatomical structures like it But a cross section taken through the rigid chamber K looks exactly like the vortex veins The rigid chamber K is like the walls of the sclera and elastic tube E – the thin walled vein – is surrounded by pressurized fluids – the lymphatic

With the model we repeated the experiment in which the effect of renal vein occlusion on renal blood flow was determined Fluid flow through the model was ascertained while a tourniquet at point O was being slowly tightened Pressures at B were also measured It was found that the tourniquet could be tightened considerably before the rate of outflow was influenced In fact the flow out of the system was not influenced until the pressure at point B equalled pressure T At first glance this seems paradoxical because here is a system with a large resistance at constriction A B Then another resistance is added by constricting at O And yet as I said this second resistance does not influence flow What happens is this as constriction O increases constriction A B relaxes There is a nice reciprocal relation between the two Adding constriction O does not increase the resistance to flow because constriction A B

relaxes When the pressure at B equals pressure T constriction A B disappears at this point further constriction of 0 slows the flow The elastic tube E is almost lying in this experiment Or stating it conversely our postulated constriction in the vortex vein may fluctuate greatly due to simple mechanical factors

Returning to the eye the illustration in Fuchs article shows structures which appear morphologically very similar to those in the model a rigid outer wall of sclera and a thin walled vein exposed to a collapsing pressure from the perivascular lymphatic The logical place for a constriction to form is just before the vein wall becomes thick as it emerges from the sclera In the illustration the accompanying lymphatic appears to open into the episcleral tissue But if the postulated system is at work the lymphatic cannot be open It should stop just where the vein wall thickens for we presume that it carries the intraocular pressure of 25 mm Hg down to the veins exit and imposes a constriction just before exit

I am unable to determine from the older accounts what actually happens in this region It must be examined in more detail But it is known that the intraocular venous pressure is about 27 mm Hg and that the pressure in the vortex vein drops abruptly so that it is only about 18 mm as it leaves the eye The same change takes place in the arcuate interlobar region of the kidney

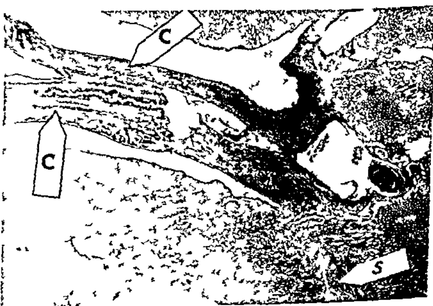


FIGURE 37 Section of interlobar region of kidney

Figure 37 shows a section of dog kidney taken through the inter-lobar trunk. Artery and vein are apparent. Lining the trunk is a band of connective tissue (C) that is unexpectedly heavy. It runs up into a spur (S) and follows the *fornices* of the minor calyces. Figure 38 was taken a little higher in the kidney. An arcuate vein and artery are apparent as is the fornix of a calyx. Between the

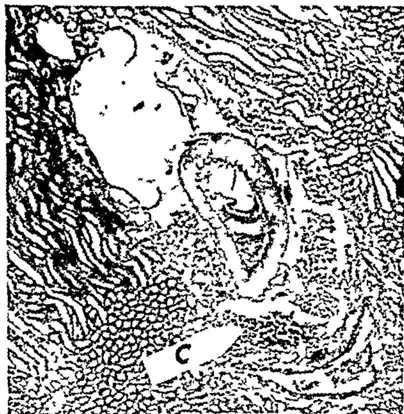


Figure 38. Section of arcuate blood vessels of kidney

calyx and the artery is a continuation of the band of connective tissue shown in Figure 37 (C). It looks like a sort of support

cu

I

hi

m

FIGURE 39 Section of juxtamedullary blood vessels of kidney



FIGURE 40 Renal artery with perivascular lymphatic

lymphatic (L) and above this the vein (V) This arrangement is characteristic of renal lymphatics they run between the artery and vein Here the lymphatic does not surround the vein as it does in the vortex veins of the eye Nevertheless it is entirely possible that the pressure inside the lymphatic may partially collapse the vein and hence put in the kind of constriction that our theory demands

I shall present the next Figure briefly primarily because I am anxious to get some hints about its meaning Figure 40 shows a section still higher above the arcuates The artery appears to be completely surrounded with lymphatic This same sort of perivascular lymph channel is seen in the ocular arteries Dr Oliver what is that lymphatic doing there?

Oliver I don t know that those ill defined spaces are lymphatics

Grafflin Are they lymph channels lined with endothelium or are they interstices in connective tissue?

Swann They look like interstices to us Peirce(28) has described the same kind of structure

Fremont Smith Aren t they very similar to the perivascular spaces so called in the central nervous system?

Oliver I don t think these spaces in the kidney are as definite structures as those in the central nervous system

Dock The interlobar veins you are speaking of are not out in the pelvis but far up in the middle of the kidney The vena cava the adrenal veins and the renal veins have considerable longitudinal muscle in their walls and wherever there is longitudinal muscle the vein is not easily collapsed by outside pressure Perhaps the fall of pressure between the arcuate and interlobar veins may be accounted for by this fact These muscles would keep the vessel stiff and prevent collapse by the high interstitial pressure

Grafflin Have you seen longitudinal muscle extend up that far in the veins?

Dock I thought I had

Grafflin I have seen longitudinal muscle in the renal veins but I don t know how far it extends

Dock That is what we need to know The resemblance between the renal parenchymal and subcapsular veins and the medullary vein of the adrenal with its very heavy longitudinal muscle bundles is pointed out in Von Mollendorf's *Handbuch der mikroscopischen Anatomie des Menschen*(29) In Vol VII Part I Figure 119 on page 132 and Figure 124 on page 136 show arcuate and stellate (subcapsular) veins which have longitudinal bundles occupying one third or less of the circumference In the lobar veins the entire

V
L
C

FIGURE 39 Section of juxtamedullary blood vessels of kidney

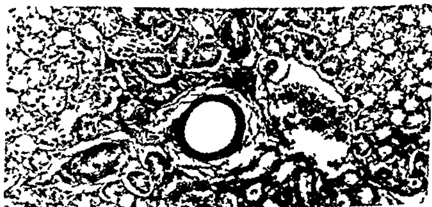


FIGURE 40 Renal artery with perivascular lymphatic

The coefficient of correlation between the two variables is 85 which we consider good for this sort of biological work When the blood pressure increased 1 mm Hg, the renal interstitial pressure increased 22 mm

It has been known for a long time that the kidney is inflated with blood In a very real sense, with our present technique of measuring interstitial pressure, we are measuring the amount of inflation of the kidney In diuresis, for example, the kidney increases considerably in size, its interstitial pressure also increases greatly, as Dr Winton has shown(1) The arterial pressure also inflates the kidney(31) Table IV shows the amount of inflation In this experiment, a hemostat was set on the renal pedicle of anesthetized dogs Then the kidney with its attached blood vessels was removed the hemostat unclamped and all the trapped blood allowed to flow out into a beaker With normal blood pressures, the quantity of blood which flows out is about 20 per cent of the kidneys weight The organ is obviously considerably inflated with blood

TABLE IV

Inflation of the Kidney by the Blood Pressure

No	Blood pressure mm Hg	IRP mm Hg	Kidney weight (engorged) gm	Blood draining ml	ℓℓ blood
1	163/119	21	30 1	6 4	21
2	158/110	30	38 2	8 0	21
3	143/105	21	43 5	12 0	28
4	136 101	19	24 5	4 1	17
5	150/100	18	36 9	9 6	26
6	127/72	25	52 2	8 1	16
Means	146/101	22	37 6	8 0	22

I suspect that this inflation is primarily in the peritubular capillaries and not in the glomeruli for the following reasons We may compute the volume of the glomerular vascular bed to be about 4 milliliters The average diameter of the renal corpuscle is taken to be 0 2 mm and, it is assumed there are a million of them Assuming that they are spherical their total volume is about 4 ml This volume is not all blood, of course but on the other hand, each

circumference has such a layer, just as in the vena cava inferior and the adrenal medullary vein

Bradley Is there any difference between the interstitial pressures in the cortex and the medulla of the kidney?

Swann We have gone back frequently to investigate that question When we insert the needle very carefully into the cortex only, we find there the same pressure as appears in an ordinary insertion With our present technique, we are not able to demonstrate any difference

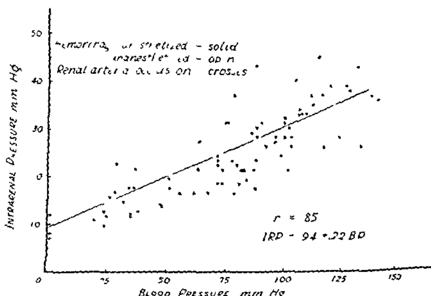


Figure 41 Effect of lowering blood pressure on intrarenal pressure Printed by permission from Swann H G et al The influence of arterial pressure on intrarenal pressure *Am J Physiol* (In press)

Figure 41 shows the effect of lowering the blood pressure on the renal interstitial pressure(30) We reduced the animal's blood *pressures by hemorrhage measuring the changes in interstitial* pressure all the while We used anesthetized dogs and unanesthetized dogs as well, because I do not trust data from anesthetized animals too far We also occluded the renal artery in seven dogs and then measured the interstitial pressure In all groups, *the data* were consistent The renal interstitial pressure (IRP) is related to the blood pressure (BP) thus, the units being in millimeters of mercury

$$\text{IRP} = 9\ 4 + 22\ \text{BP}$$

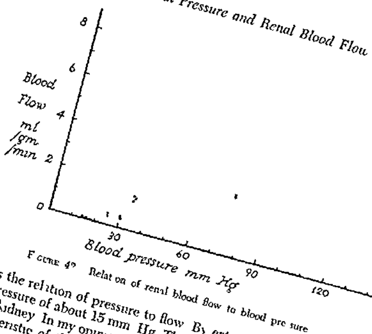


Figure 42 Relation of renal blood flow to blood pressure

shows the relation of pressure to flow By extrapolation flow ceases at a pressure of about 15 mm Hg This is called the yield pressure of the kidney In my opinion it can be ascribed not to some peculiar characteristic of the blood such as pseudoplasticity but rather to a simple dimensional factor i e collapse of the capillaries by the interstitial pressure Before the capillaries can open this pressure must be overcome Hence no flow occurs until the blood pressure reaches 12 to 15 mm Hg

Winton Are these steady state measurements?

Swann Yes

Winton How do you measure the blood flow?

Swann By means of a graduate and stop watch

Winton How do you lower the blood pressure?

Swann The renal vein was cannulated and its blood led into the jugular This system was tapped periodically and all its blood drained into a graduate for five or ten seconds In this way the flow rate was measured The blood pressure was lowered by putting a tourniquet around the aorta above the renal arteries By graded twisting of the tourniquet the aorta was partially occluded and thus the blood pressure lowered to any given level

Dr Winton has already called attention to the linear relation of flow and pressure at low pressures But at higher pressures the flow tends to taper off Figure 43 is the record of the nine individual dogs the relation of flow to blood pressure being shown by circles

corpuscle has perhaps shrunk with death and tissue fixation The 4 ml figure represents I think approximately the quantity of blood in all glomerular tufts A man s dead kidney weighs about 150 gms and at a normal blood pressure it is probably inflated with 20 per cent of its weight in blood or about 30 ml of blood We can account for 4 ml of this blood in the glomeruli Another fraction is in the large blood vessels — as a guess not more than 10 ml But this leaves a large volume of blood unaccounted for

I feel sure that this extra volume is in the peritubular capillaries and that they are greatly inflated with blood Returning to the eye for a moment its capillaries are very large Instead of being 10 micra in diameter as they are in most tissues they are about 30 Some run up to 100 micra in diameter so that ten red cells can march through them abreast The same situation holds I suspect in the kidneys its peritubular capillaries are widely inflated with blood This after all is a very desirable state of affairs

The peritubular capillaries in the kidney are extraordinarily long capillaries Most of the body s capillaries are roughly a half millimeter long while those of the peritubular network are 10 20 or even 30 mm long This is 20 or even 100 times longer than most capillaries Now the resistance of a 30 mm capillary if it had a bore of only 10 micra would be very great But because they are so inflated with blood their bore is greater and their resistance is reduced

Oliver But the peritubular capillaries are a network and there are all sorts of anastomoses I don t see how any specific length can be assigned to a particular capillary

Swann Certainly one capillary runs from the efferent arteriole all the way down to the end of Henle s loop a distance of approximately 30 millimeters

Oliver Yes but that seems to me a very exceptional capillary most of the efferents break up almost immediately into a network

Swann They are far longer than a half millimeter

Oliver No many of them are not

Winton I am sure Dr Swann must be right about the blood inflating the kidney in this way because if vasoconstriction is induced with adrenalin or if the arterial pressure is reduced by 60 mm Hg or more urine runs back into the kidney when the ureter pressure is raised as if there were a variable space in the kidney which is immediately accessible to the blood

Swann We measured blood pressure blood flow and interstitial pressure simultaneously in nine nembutalized dogs Figure 42

pressure rises rapidly, e g , No 30 The relation varies from dog to dog But the average of the slopes is 22, the same as obtained in the previous experiment

Each dog is evidently a law unto itself in this situation, the range of slopes being from 09 to 34 It tempts us to speculate that the hypertensive diathesis is due to this particular relationship if the interstitial pressure rises rapidly with a given rise in blood pressure as it does in No 30, the pregnant dog it would cause an unexpectedly great resistance to blood flow The hypertensive diathesis, then, would be one in which a normal blood pressure is opposed by exceptionally high interstitial pressures To get adequate renal blood flow, therefore, the hypertensive's blood pressure would have to be high

But unfortunately for this hypothesis as the blood pressure increases, the rate of change in interstitial pressure and in blood flow is not related If our hypothesis were correct, the two rates of changes would be inversely related one would expect the flow to increase greatly with a given blood pressure change if with this same blood pressure change the interstitial pressure increased only slightly Table V shows the rates of change in interstitial pressure, arranged in order of increasing values The rates of change in

TABLE V

Relation of Increase in Interstitial Pressure to Increase in Blood Flow

Dog No	Increases per mm Hg rise in blood pressure in	
	Renal interstitial pressure mm Hg	Renal blood flow ml /gm /min
73	09	059
44	14	072
32	17	070
64	19	072
77	22	046
48	25	046
82	27	068
79	28	062
30	34	081

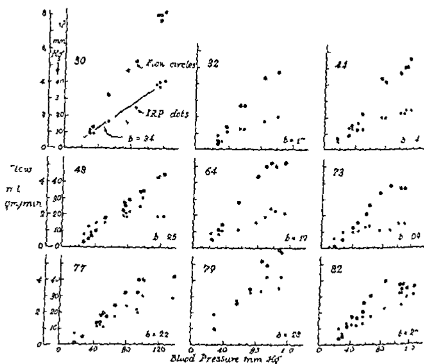


FIGURE 43 Relation of renal blood flow, blood pressure and interstitial pressure (IRP) in 9 dogs The slopes (b) of the flow interstitial pressure relationship are indicated for each dog

and the relation of interstitial pressure to blood pressure being shown by dots An exceptionally high flow rate was observed in one dog, No 30 The dog was pregnant, which perhaps explains it

In this experiment, we found a point of inflection in the flow pressure curve in only five of the nine dogs and in only four — Nos 64 73, 77 and 82 — does it "elbow over" sharply But the important thing which I wish particularly to call to your attention is the fact that even when it does elbow over sharply, as in No 82, the interstitial pressure does not rise abruptly If it did, we would say that it gives rise to the sudden increase in resistance which is responsible for the relatively slow flow at this point of high blood pressure But it does not

Figure 43 also shows the slope (b) for each curve relating blood pressure to interstitial pressure The units are millimeters rise in interstitial pressure per millimeter rise in blood pressure In some cases e g No 73 the interstitial pressure rises very slowly with a given change in blood pressure But in other cases, the interstitial

Certainly the glomeruli maintain a constant rate of filtration despite changes in pressure which cannot be easily explained on a mechanical basis I should think that the arterioles adjust their setting in response to the pressure change

Winton The kind of vasoconstriction being invoked seems to me very difficult to bring into the story since the phenomenon persists in the kidneys in the presence of a concentration of poisons which prevents any reaction of plain muscle It also persists at 3 C at which mammalian smooth muscle is not a very reactive tissue

Dock It might be worthwhile to perfuse dead kidneys with kerosene which does not cause edema of the vessel wall

Winton I have tried petrol not kerosene The surface tension oblem rather complicates things

Shannon May I ask Dr Swann about one point that has to do h what he calls his crowbar technique It seems likely that n such a crowbar is put into renal substance and so fragments ascular system a pool is created to which both the pressure in the arterial system and the pressure in the tubular system have access The fact that recorded pressure is reproducible does not appear to be too important The units are so small that each time such a pool is created access would be had to these two systems in the interstitial tissue Pressures so derived may not be representa tive of those in the interstitial fluids themselves

Swann That is a just criticism If we had only the data obtained with the transduced equilibrium method to support our position that position would be somewhat shaky But the data on arcuate enous pressures — a completely different technique — confirm the ther data well Hence I do not think that the factors you have scribed vitiate the results obtained with the transduced equi rium method

I might say that I wish that the arcuate and interlobar veins were more accessible Cannulating them is difficult and unpredictable In only about half of our attempts do we succeed in cannulating the arcuate vein

Shannon Is there venous spasm?

Swann No We can always withdraw the catheter easily We can also draw blood out of it easily

Winton You find a pressure in the large vein of about 15 millimeters of mercury In my nembutalized dogs the venous pressures are much lower than that and I wonder if you are getting edema due to high venous pressure

Swann That pressure may be influenced by the position of our

flow, given the same blood pressure change, should run in the reverse order *But they do not, they are spread in random order* through the Table

I wish to add one point more At low and medium blood pressures the renal blood flow is linearly related to the blood pressure In most tissues, however, blood flow is exponentially related to pressure, the curve being convex to the pressure axis I think that the reason for the linearity of the kidney curve (that is, in its lower portion) lies with the interstitial pressure The latter rises with rising blood pressure, a rising blood pressure *per se*, therefore, causes an increase in the circuit's resistance The situation is similar to an incandescent light the greater the pressure or EMF, the hotter the filament becomes, the hotter the filament the more the resistance to current flow At medium high blood pressures *therefore, renal blood flow* is *disproportionately slowed by the* high interstitial pressure But, as I have already indicated the behavior of interstitial pressures at the higher blood pressures can not be used to account for the 'elbowing over' of the renal pressure flow curve

Dock It seems to me that decapsulation(32) might simplify the problem a good deal

Swann If the capsule were maximally stretched at the point of inflection of these flow pressure curves, then any added increment of blood pressure should cause a very high interstitial pressure But this does not happen Consequently, I do not think the capsule is involved in the elbowing over' of the curve

Winton I have measured the intrarenal pressure before and after decapsulation a number of times, and it is approximately halved by decapsulation The renal substance itself is quite rigid If one of the two branches of the renal artery that goes into the kidney is tied off the circulation through that half ceases The kidney shrinks and the interstitial pressure in the surviving part is usually about halved

Dock But that makes no difference to your flow pressure relationship

Winton Oh no it still has the same form

Swann I agree with you about the effects of decapsulation Even the high interstitial pressure of diuresis is about halved when the kidney is decapsulated

Selkurt In spite of what has been said I am inclined to think a vascular response accounts for the change in the pressure flow curve, although we cannot go on to say what the mechanism is

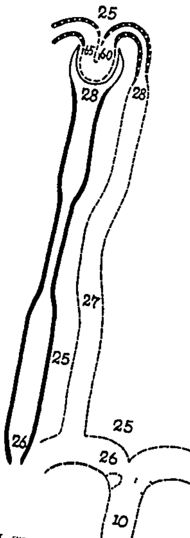


FIGURE 41 Hydrostatic pressures postulated to be operating in the kidney The vascular portion is dotted

working almost unopposed It is drawing fluid inwards with a essure of 25 mm less the net hydrostatic pressure of 2 mm ving the net pressure inwards to be 23 mm Hg Here may be the mechanism for reabsorption of 99 per cent of glomerular filtrate We may consider that the nephron is a

animals They are prone, and after the dissection the kidney hangs down from the vena cava on, as it were, a stalk That would account for some of that 15 mm Hg of pressure

Winton As far as I could see when I was privileged to watch you at work in Texas our techniques are not so very different but of course, with nembutal venous pressure can go up, and I don't know whether your dose and mine were the same or whether the same doses produce the same effects in different climates

Selkurt We also found about 7 5 mm as the average renal venous pressure in dogs, and that was the end pressure on forcing in the cannula

Chinard If your figures are correct, Dr Swann, wouldn't they lead to a revision of the generally quoted estimate of about 10 mm Hg for the glomerular pressure? They would be boosted up above 25 I think, to get any fluid down the tubule, which would make the relationships generally considered to be acceptable quite out of line?

Swann Yes, that is right

Figure 44 shows the several hydrostatic pressures that we think are operating in the kidney We suppose that the pressure drop through the glomerular tuft is from 65 to 60 mm Hg, and that then there is a pronounced drop through the efferent arteriole to 28 mm Following that there is only a small pressure drop all the way through the peritubular capillaries and into the venules But as the blood flows from the arcuate veins into the interlobars it passes through the constriction we have postulated for this region and therefore its pressure suddenly drops from 26 to 10 mm Inside the tubule the pressure is postulated to be just above 25 mm Hg through all its length Surrounding all of these structures is the interstitial space with a pressure of 25 mm Hg These are hydrostatic pressures One interesting consequence of our theory is this in the peritubular capillaries the hydrostatic pressure from the heart is almost completely balanced by the interstitial pressure The net hydrostatic pressure will be 27 minus 25 mm Hg or 2 mm outwards

Winton Do you suppose those vessels are so rigid that the pressure from the interstitial fluid is not transmitted to the contents of the vessel?

Swann We have to assume that the vessels involved are freely collapsible

Inside the capillary, therefore, with its net hydrostatic pressure outwards of only 2 mm Hg the plasma protein osmotic pressure

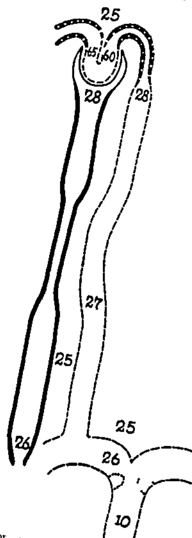


FIGURE 41 Hydrostatic pressures postulated to be operating in the kidney. The vascular portion is dotted.

is working almost unopposed. It is drawing fluid inwards with a pressure of 25 mm. less the net hydrostatic pressure of 2 mm. leaving the net pressure inwards to be 23 mm. Hg.

Here may be the mechanism for reabsorption of 99 per cent of the glomerular filtrate. We may consider that the nephron is a

specialized example of Starling's classical capillary the glomerulus is the arterial end, the tubule is a specialized interstitial space, and the peritubular capillaries are a specialized venous end In the latter, reabsorption of glomerular filtrate is so complete because the filtrate is drawn into the peritubular capillaries by the virtually unopposed plasma protein osmotic pressure *

Winton I am afraid I cannot follow that argument at all You have a tissue pressure, and you have been talking about this tissue pressure as if it were transmitted to the contents of the tubules Similarly, it is transmitted to the contents of the blood vessels so that pressure is equally applied on the inside and the outside of the vessels That could not possibly be brought into an argument about the transfer of fluid across their walls

Swann The capillary is being inflated, as it were, against the interstitial pressure

Winton On the inside?

Swann Yes the inflating pressure is pushing outwards If you oppose the hydrostatic pressure from the heart with the tissue pressure the net hydrostatic pressure is 2 mm Hg This leaves the blood oncotic pressure virtually unopposed to draw fluid back into the capillary

Winton If we have these high interstitial pressures—and I think Dr Swann conceives of them as obstructing outflow of urine—that means that the pressure on the ureter side of the glomerular membrane must be pretty high There must also, presumably, be a pressure fall across the membrane I wonder whether Dr Pappenheimer would be able to give us any kind of suggestion from his experience with membranes as to what that pressure fall might be

Pappenheimer I have just taken your figures from the literature that is about 70 as the pressure on the glomerular side, and subtracted the protein osmotic pressure from this value

Winton But the actual dynamic movement of the whole glomerular filtrate across that membrane must involve some viscous resistance, mustn t it?

Pappenheimer It could conceivably be very low, much lower than we all suppose, so it would not be impossible, by any means, to have a very small pressure drop across the glomular membrane

* To oversimplify in this view the renal unit is an efficient filter at one end (the glomerulus) and an efficient osmotic pressure cell at the other end (the tubule with its capillaries) Associated with the osmotic pressure cell are the cellular metabolic processes which guide differential reabsorption and secretion

Oliver There still remains the mystery of how the urine can flow down the tubule Have you solved that one?

Shannon Dr A N Richards studies in the amphibian kidney several years ago would indicate that there is adequate pressure at least in the frog to provide for the forward movement of fluid

Bott Hayman(34) made most of the measurements on frogs Glomerular capillary pressures showed great variation but averaged about 15 mm of mercury

Pappenheimer I should like to put the emphasis more on peritubular capillary absorption For years, people have argued and worried about glomerular filtration but exactly the same problems are involved in peritubular capillary reabsorption Practically the same volume is reabsorbed as is filtered in the glomerular capillaries but the only known force available for this absorption is the osmotic pressure of the proteins flowing through the peritubular capillaries This protein pressure sets a top value for the pressure difference required to produce flow (absorption) across the peritubular capillary membranes

Mudge It must be remembered that a major factor in the movement of solute from tubule to capillary is the concentration gradient established by the active transport of electrolytes

Pappenheimer We are talking about peritubular capillaries not tubular cells Capillaries from what we know of them act pretty much as inert membranes that is they cannot supply energy to produce concentration gradients

Darrow One may consider the transport of water and electrolyte out of the tubules is involving energy but from the peritubular capillary spaces water and electrolyte are transported back into the blood by the same mechanism as from other interstitial spaces

Berliner At the interstitial side of the tubule cell involved in active transport an appreciable concentration gradient of the particular substance being transported must be built up

Pappenheimer But wouldn't that tend to draw water out of the capillaries and back into the interstitial fillings?

Selkurt That is right

Berliner Some concentration gradient must be present to get things back into the capillaries

Pappenheimer I would suspect from the results discussed earlier that no appreciable concentration gradients of small molecules exist across the peritubular capillary membranes any more than across glomerular capillary membranes

Mudge That depends on the experimental set-up The sodium concentration in the reabsorbate during an osmotic diuresis becomes 50 to 70 per cent greater than the concentration in the tubule or the glomerular filtrate

Winton But that does not affect the concentration gradient across the capillary wall

Pappenheimer No, that's right, and what I simply wish to point out is that all the problems which we have thought about for years in connection with glomerular filtration, we have equally to face in peritubular absorption

Berliner Of course, you have, presumably, a much bigger surface involved here than in the glomeruli

Pappenheimer Do you, really?

Oliver It must be much larger than the surface of the glomeruli Putter once made a theoretical estimate of the various surfaces in the kidney and elaborated a theory of renal function on the basis of it, the so called "Drei-Drusen" theory(35) Without accepting all its implications, we at least admitted the importance of surfaces in renal interchanges and showed how they vary in certain abnormal kidneys(36)

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PHYSICAL FACTORS IN RELATION TO ELECTROLYTE AND WATER EXCRETION*

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INTEREST IN THIS work was aroused by Merrill in 1946(1) when he demonstrated that the sodium retention in edema of heart failure might be the result of impairment of renal blood flow and a reduction in glomerular filtration rate. The idea was put forward that the decrease in sodium excretion and the consequent retention was the result of reduction in glomerular filtration rate. As stated, this is an oversimplification because it is obvious that it implies that the excretion of sodium would fall in proportion to the change in filtration rate. As Merrill's data and the subsequent data of Mokotoff in 1948(2) show, the decreased excretion of sodium is much more rapid than the corresponding and correlated change in filtration rate.

The statement which elaborates on this and which attempts to explain the mechanism has been put somewhat as follows: when the load of sodium to the tubular cells is reduced, these cells reabsorb the load more efficiently. But this seems to me an evasion of the real problem because it takes for granted certain fundamental concepts about the sodium mechanism which to my knowledge are certainly far from settled at the present time. Furthermore, it seems to ignore certain biochemical and physical factors which may be a part of this so called more efficient reabsorption with reduced load.

It was to re-evaluate these functions and interrelationships that we started our studies in dogs and all data given will be on those animals. All experiments were done under anesthesia, either chloralose or pentobarbital.

Very early in our work we began by attempting to assess the effect of altering load and we approached it logically by a reduction in glomerular filtration rate. Table VI shows a representative experiment of this sort and illustrates some of the fundamental factors in which we became interested. This relates the excretion

* Experimental work cited in this report was supported by grants from the U S Public Health Service

TABLE VI

The Effect of Reduction of Arterial Perfusion Pressure on Sodium Excretion*

—	MBP	GFR	LOAD	UV	Reabs	% R
	mm Hg	ml /min	mEq / min	mEq / min	mEq / min	
1	120	33 9	5 33	336	4 99	93 5
2	115	35 1	5 48	334	5 15	93 6
3	97	35 4	5 48	239	5 24	95 5
4	97	35 3	5 48	200	5 28	96 3
5	82	32 4	4 92	067	4 85	98 6
6	80	33 0	4 96	049	4 91	99 0
7	68	30 8	4 59	022	4 57	99 5
8	72	30 0	4 49	018	4 47	99 5
9	61	19 1	2 85	004	2 85	100 0
10	60	19 4	2 89	001	2 89	100 0
11	119	30 6	4 60	052	4 55	98 8
12	117	31 5	4 72	063	4 66	98 8

* Pressure was decreased by aortic constriction Data are for one kidney only

of sodium in milliequivalents per minute and the filtration rate, measured by the creatinine clearance, to the alteration in perfusion (arterial) pressure These measurements were made in dogs which had a constriction around the aorta, just above the renal artery As you can see the pressure was decreased in stages and then restored to control This at first brought about no change in filtration rate As a matter of fact, in the second stage, filtration rate was even slightly higher than the control on the average Filtration slightly decreased in the second stage of reduction of pressure, and finally, it fell rather rapidly at low arterial pressures

It is interesting that the excretion of sodium (UV) begins to diminish almost immediately and goes down very markedly until it practically vanishes from the urine when perfusion pressure is lowest, then rises somewhat on return of the pressure and partial restoration of filtration rate The interesting thing about this experiment —

and there are others which show the same thing — was that the sodium excretion began to diminish even though the filtration rate remained up raising the question of secondary or auxiliary factors which might be concerned in the phenomenon of sodium excretion. Two possibilities in terms of hemodynamic factors must be considered. First the excretion of sodium may be related to change in perfusion pressure. The second is the possibility that the compression of the artery reduced the pulse pressure and that this factor might in some manner modify the excretion of water and sodium. The data on the urine volume are not presented here but the changes in urine volume are almost identical with the changes in sodium excretion percentagewise. They drop off as the sodium excretion drops off. Concomitant with reduction in excretion it can be seen (last column) that the percentage of the load which is reabsorbed increases to about 100 per cent.

The question arose whether the trend shown in Table VI is the result of the specific experimental procedure or is it some nonspecific trend instituted during the maneuver? We therefore began to control the experimental kidney with the opposite kidney and then reconsidered our data in terms of the reference control. This is a much safer way to analyze trends particularly since urine volume and sodium excretion are so responsive to changes in rate of saline infusion — and I might add that we did infuse saline in all our animals prior to the experimental phases to encourage diuresis and sodium excretion.

Figure 45 is a similar experiment which shows data obtained in a study of both kidneys. The solid symbols refer to the experimental kidney, the open to the control kidney. We almost invariably use the left kidney for the experiment and the right for the control. This experiment was done with a tourniquet around the aorta between the renal arteries so that the perfusion pressure was lower in the kidney below the constriction. (Measured from the femoral artery.) The control pressure was measured above the constriction from the carotid artery. The pressure in the control kidney remained essentially constant whereas the pressure in the opposite kidney was decreased to 88, then 75, then 60 mm Hg and then restored. These are averages for the paired periods which we obtained invariably at each step.

Here again it can be seen that there was at first no change in the creatinine clearance even though the pressure was reduced from 115 to 88 mm Hg. We ascribe this to "renal autonomy." While the creatinine clearance stays constant the excretion

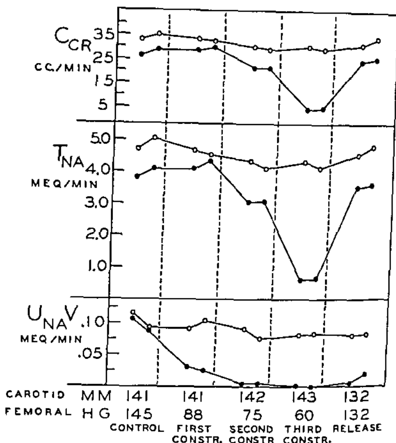


FIGURE 45 Effect of reduction of arterial pressure on glomerular filtration rate (C_{CR}), tubular reabsorption of sodium (T_{NA}), and sodium excretion ($U_{NA}V$) Experimental kidney in solid symbols, control kidney in open symbols

sodium falls very markedly with the reduction in perfusion pressure It remains reasonably constant on the control side It goes down further in the second stage, but this is complicated by a reduction in filtration rate which is further exaggerated in the next stage, where it remains quite low, and the sodium practically disappears from the urine On release, the excretion does not come up to control This is quite characteristic of this procedure, where a full recovery was not obtained in the process of restoring perfusion pressure We are not certain how to explain it In this animal (Figure 45), the perfusion pressure did not come back to the original value However, it did equal the pressure of the control kidney whose salt excretion remained up Possibly, the load itself

played a role here, since the creatinine clearance apparently did not return quite to control It is very likely that some other unknown factor was set into play which would explain this failure of recovery The point I want to bring out with Figure 45 is that the trend shown in the previous experiment is not a nonspecific thing, but is actually the result of constricting the artery and reducing the perfusion pressure And here, too, we find that this change in excretion can come about without decreases in load The rate of sodium reabsorption (in milliequivalents per minute (T_{Na})) goes up slightly at first as the excretion falls

One factor which may aid and facilitate the influence of absolute change in load on excretion of salt and water when filtration rate is altered may be the absolute level of the renal arterial perfusion pressure But we still do not know what the effect of the concomitant reduction in pulse pressure is, since as soon as one begins to drop pressure and constricts the aorta the pulse pressure is reduced as part and parcel of the mechanism for reducing the mean pressure In 1910 Dr Hooker(3) studying isolated perfused kidneys, concluded that the reduction in pulse pressure reduced renal blood flow and urine volume and in 1913 Gesell(4), studying the same problem in intact dog kidneys, reached somewhat the same conclusion in terms of the effect of the reduced pulse pressure on urine volume and chloride excretion but disproved Hooker's notion that a reduction in pulse pressure would alter the blood flow Thus it seemed important either to rule out this factor or to consider it as a serious possibility

We used a somewhat different technique in the next series of animals This technique (Figure 46), was to perfuse the left kidney by an external circuit from a carotid cannula (A) through an

ternal circuit Other details of this hookup are the outflow cannula for sampling the renal blood (C) a compression chamber which could be opened if it was desired to reduce the pulse pressure (F), an optical manometer (E) to register the pulse pressure directly A third alternative possibility in later experiments, when it was desired to elevate the mean pressure above the dog's own pressure, was to connect in the pump shown at the left This is a pump which has flexible elastomold tubing compressed 120 times a minute by a rotating cam (B) the stroke volume can be varied by pushing the position of the cam to and away from the tube Flap valves

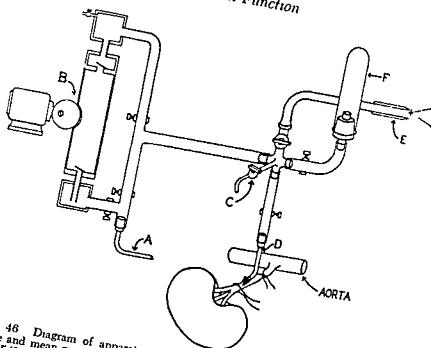


FIGURE 46 Diagram of apparatus for experimental modification of arterial pulse pressure and mean pressure Reprinted by permission from Selkurt, E E *Circulation* 4, 541 (1951)

keep the blood moving in one direction so that this offers a way of increasing the mean pressure and maintaining a pulsatile pressure

Our first approach was to find out if the pulse pressure decrement would have any influence on renal function at the dog's normal arterial pressure, and Figure 47 shows a representative experiment of a group of six animals in which this point was examined The data given include the para aminohippurate clearance, the creatinine clearance the excretion of sodium, and the urine volume Below are given the pressure relationships, the pressures in the perfused kidney, measured by the optical manometer, and the control pressure, a mean pressure taken from the carotid You can see at a glance that there was no apparent effect attributable to the reduction in pulse pressure from 36 to 10 mm Hg on any of these functions when the perfusion pressure was kept constant

Might not this change in pulse pressure have an influence on kidney function if the pressure were lowered? This was considered because some of the data that Gesell presented were in animals which had a low mean pressure, and so the possibility was present that perhaps the effect would obtain when the mean pressure was decreased and the pulse damped out

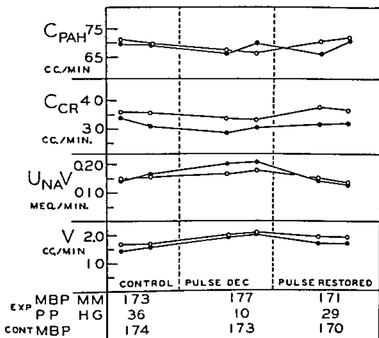


FIGURE 47 Influence of reduction of pulse pressure with constantly maintained mean arterial pressure C_{PAH} clearance of para aminohippurate, V urine volume Experimental kidney in solid symbols control kidney in open symbols

Figure 48 is a representative experiment Below are given the pressures of the experimental kidney Following the control stage, the pressure was decreased to 89 mm Hg, and held there for the entire experimental phase Now, as the tubing is compressed to reduce the mean pressure the pulse pressure is reduced It fell in this study from 49 to 14 mm Hg In the next stage, the pulse pressure was more or less eliminated by connecting in the compression chamber Then the pulse pressure was restored, still maintaining the same reduced mean pressure and, finally, both the mean and pulse pressures were restored

Here (Figure 48) are shown the typical data for para aminohippurate clearance, creatinine clearance excretion of sodium, and urine volume of the experimental kidney (solid) and the control (open) As can be seen there is a gradual downward trend of the para aminohippuric acid clearance and often the creatinine cl ance, but not to the same extent It is obvious, too that the

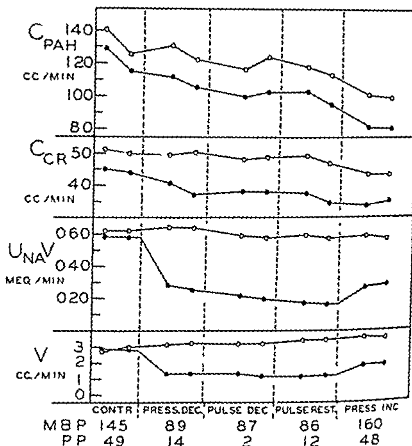


FIGURE 48 Influence of reduced pulse pressure after reduction of mean arterial pressure Pressure given in mm Hg below for experimental kidney only

ference of the PAH clearance between these two kidneys changes little, if any

The creatinine clearance falls from about 43 ml per min down to 41, and finally down to about 38 ml per min during the first stage of pressure reduction Subsequently, there is very little change as we modify the pulse pressure The same is true of the para-amino hippurate clearance which has a trend which is uninfluenced by a subsequent moderation of the pulse pressure

Sodium excretion falls off rather markedly from nearly 0 6 mEq per min to about 0 3 when the profusion pressure is reduced The urine volume on the experimental side shows a proportional reduction Here again there is no change in the trend of the sodium excretion as a result of altering the pulse pressure, nor is there any

influence on the trend of the urine volume The important thing to point out is that the same marked fall in sodium excretion and urine volume occurs as the perfusion pressure is reduced It must be pointed out that the load was simultaneously somewhat reduced in this experiment

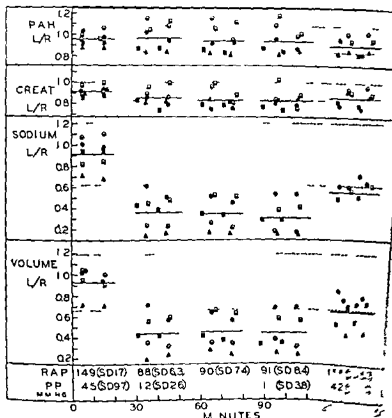


FIGURE 43 Summary of five experiments similar to representa... Figure 48 Data given here as ratios of experimental to control kid...

Figure 49 summarizes a group of five animals which ... in the same way Here the data are given only as ... right to correct for any general systemic nonspecific ... might be operating on kidney function The same ... are noted para aminohippurate and creatinine cl... tion of sodium and urine volume Below are give...

They do not differ much from the representative experiment (Figure 48) and show about the same response in terms of absolute changes in mean pressure and pulse pressure The collection periods are given individually, with the dotted lines at plus or minus two standard deviations, and the solid lines representing the average of each group in each stage You can see that, on the average, there is no effect of reduction of mean pressure or subsequent modification of pulse pressure on the para aminohippurate clearance, taken to represent plasma flow and, of course, indirectly, renal blood flow There is a small fall of the creatinine clearance from an average of 0 91 in the control to about 0 86, unaltered by further manipulation of the pulse pressure and restored slightly on return of the mean pressure The sodium excretion drops, as can be seen, from a control average ratio of 0 91 down to less than 0 40, and then it does not change much as the pulse pressure is modified and returns almost to 0 60 on return of the mean pressure The urine volume shows a corresponding change, very much like the sodium excretion in terms of percentile changes of the experimental from the control Looking at the average data, I think it will be agreed that the modification of the pulse pressure at reduced perfusion pressure has no effect on any of the measured functions

In two of these experiments there was no change in load or in the filtration rate on a percentage basis – the partially closed squares and circles – nonetheless, a marked fall in sodium excretion and urine volume occurred This effect can also be demonstrated in terms of absolute filtration rate, as shown earlier This observation, then, brings in very definitely the question of the possibility of secondary factors, along with changes in load, which might modify the reabsorption of sodium and water

We carried the experiment in another direction We elevated the perfusion pressure by use of the pump, and with that modified the pulse pressure in much the same manner as previously Figure 50 shows a representative experiment of this group These experiments consisted of increasing the perfusion pressure in the first stage, then trying to maintain the mean pressure and diminishing the pulse pressure in the second stage, followed by restoration In this animal the arterial pressure showed a gradual downward trend To bring out more forcibly the effect of the perfusion pressures, they are given as ratios The perfusion pressure was increased about 33 per cent above the control in the first experimental stage by connecting the pump to the circuit, then the pulse pressure was decreased

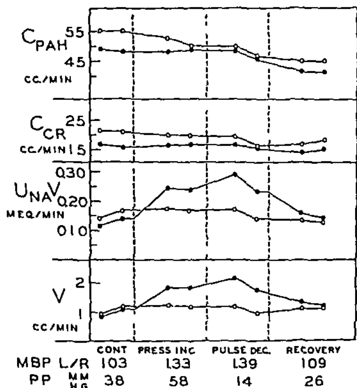


FIGURE 50 Representative experiment in which arterial perfusion pressure was elevated by means of the pump with subsequent reduction of the pulse pressure

from 58 to 14 mm The mean pressure rose to 139 per cent of control Then it was restored fairly well on recovery

It can be seen that the elevation of the mean perfusion pressure 33 per cent or in absolute terms from 120 to 175 mm Hg had no effect on the para aminohippurate clearance or the creatinine clearance in this experiment But the excretion of sodium and the urine volume increased

In the next stage in which the pulse pressure was diminished there was a momentary increase in the excretion of sodium for the first period and correspondingly for urine volume It settled down to about the same level as in the previous stage in the second period We were led to conclude by this experiment and others which I will show in the next Figure that the reduction of pulse pressure *per se* had no effect after the perfusion pressure had been elevated

Again the striking underlying influence of the perfusion pressure is evident in this experiment since the sodium excretion and urine volume went up as the perfusion pressure was increased despite reasonably constant creatinine clearances

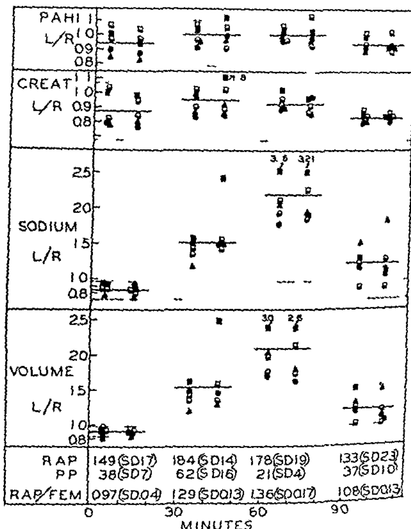


Figure 51 Summary of five experiments similar to representative experiment of Figure 50 Data are given as ratios of experimental to control

Figure 51 is a mass plot of all animals studied in this manner and given again in terms of ratios as in the earlier Figures The renal arterial pressure was first raised on the average from 149 to

184 mm Hg, then dropped somewhat to 178, and finally restored to 133 The pulse pressure was increased on the average from 38 to 62 mm Hg, then diminished to 21, and restored to 37 Below are given the ratios of pressure of the experimental kidney to the femoral pressure, used in these experiments as the control pressure for the opposite kidney They went up from about 97 to 1 29 In the second stage, the ratio increased to 1 36, mainly because the control pressure tended to go down slightly and the experimental pressure was maintained On the average, there was little change in the para-aminohippurate clearance The mean showed a slight increase and then returned There was a slight increase in the creatinine clearance (in terms of ratios) when the perfusion pressure was increased, and return upon decrease of the pressure However, in this group, as in the earlier one, we have examples where the changes in sodium excretion and urine volume occur without demonstrable change in creatinine clearance The creatinine clearance of all experiments, on the average, tends to fall in the second experimental phase, but the sodium excretion and urine keep going up The question arises is this further increase in excretion of sodium and water the result of a diminution of the pulse pressure? It will be recalled that in the representative experiment there was no effect

Pitts Dr Selkurt, may I ask a question here? What was the condition of these animals with respect to sodium loading during the course of the experiment?

Selkurt All these animals were perfused with Locke s, Ringer's, or saline for about a half hour to forty five minutes before the experiment, and a continuous infusion was carried on throughout the entire course of the experiment Our average rate of infusion was about 6 or 7 ml per min, usually of Ringer's solution

Pappenheimer How much did that raise the plasma concentration of sodium?

Selkurt It ordinarily does not raise it Sometimes the sodium level does change, but, of course, on theoretical grounds, an isotonic salt infusion would not be expected to alter the sodium plasma level That is generally true, although we have observed changes in plasma sodium even with infusion of isotonic saline, which I cannot explain

Pappenheimer Wouldn't it change the chloride a great deal?

Selkurt It is possible but we have not investigated the chloride mechanism

Pappenheimer Are these animals in progressively increasing sodium balance? In other words, are they excreting what they get

or less than what they get?

Selkurt I would say that they probably are in increasingly positive sodium balance The nonspecific factors are probably controlled by comparison of the experimental kidney with the opposite control side Although it is true that slight changes in plasma sodium do occur, and it is also true that the gradual building up of a reserve of saline in their plasma, and possibly in their interstitial spaces, results, we feel that these nonspecific factors, if they do finally touch off some hormonal mechanism, ought to act on both kidneys more or less equally

Shock Does the pump being used produce any appreciable hemolysis?

Selkurt There is no visible hemolysis in those samples

I was discussing the possibility that the pulse pressure reduction during the second experimental phase might be increasing the excretion of sodium, as indicated in Figure 51 I am inclined to dismiss this possibility because it appears that the trend instituted by the elevation of perfusion pressure is a progressive one Most of the second periods in the first phase are higher than the first Here (solid squares) is a very marked increase, for example, even though we keep the perfusion pressure and also the pulse pressure constant, and it may be that this trend continues on into the second stage Another possibility is that the ratio of perfusion pressure is higher for technical reasons in the second stage than the first one The third possibility is that reduction of pulse pressure may actually increase the excretion of sodium but we do not consider that possibility a likely one

These data, then, seem to indicate that there are auxiliary factors which may facilitate or impede sodium reabsorption since changes often occur even when the filtration rate or load remains essentially constant That raises the question of what these possible factors might be and here we must enter the realm of speculation When we vary the perfusion pressure we may do something to the rate of movement of the filtrate down the nephron and to the time relationship for reabsorption of sodium and water That seems not to be too likely unless the observed constancy of filtration rate, despite changes in pressure, occurs in association with the opening and closing of nephrons If that is the case, then it might be possible that closure of nephrons during elevated pressures would hold the total filtration rate down but would cause the filtering force in open nephrons to increase and facilitate the movement of the filtrate and tubular urine at a faster rate

However we believe that the more or less constant filtration rate is the result of adjustment of the arterioles in the kidney that it probably goes on proportionately in all nephrons And other general evidence of course tends to favor the idea that the autonomy of the circulation in the dog results from varying degrees of change of the arterioles The idea of opening and closing nephrons is not generally accepted Therefore if the filtration rate is maintained constant by the adjustment of arterioles the filtration pressure should be kept constant and so also the movement of tubular urine

The other possibility since we have dismissed the factor of pulse pressure might be changing interstitial pressures in the kidney The facts you well know by now If the interstitial forces play a role in furthering or hampering the movement of fluid and solutes from the tubular cells to the blood then the intrarenal pressure must change with the perfusion pressures since sodium and water excretion are related to the mean perfusion pressure We would have to expect that the intrarenal pressure would vary with the arterial pressure

Pitts How are you using the term intrarenal pressure"?

Selkurt I use it in the loosest sense possible I did imply or meant to imply the pressure in the interstitial spaces which do not exist according to Dr Oliver so we are really in a bad way all around But bear with the idea for a moment and we will return to this problem toward the end when we have seen some more data and battle it out at that time

But I do want to say that Gottschalk(5) believes that the renal interstitial pressure increases when the arterial pressure is above 140 mm Hg and decreases below 40 You have seen the data of Dr Swann and you know that he feels that the intrarenal pressure that is the renal interstitial pressure does change throughout the entire range of variations of arterial pressure If that is true then we might have some basis for speculation in terms of the forces involved either favoring or impeding the movement of ions and fluid between the tubular structures and the capillary system We would explain the increased excretion of sodium and water with the elevated perfusion pressure in the face of a constant load by an increase in the pressure existing in the renal interstitial spaces which may not exist At any rate if that mechanism is the true one it has a certain logical appeal Since we are setting up a force which impedes the free movement of ions and water it may explain at least in part the phenomenon of increased sodium and urine loss at elevated pressure The reciprocal effect would be noticed when

we decrease perfusion pressure

The difficulty with this hypothesis – and it is nothing more than an hypothesis – is the fact that when venous pressure and ureteral pressure are elevated, there is no question about the fact that there is an increase in intrarenal pressure And yet, under those circumstances, sodium and water are retained rather than lost as when the arterial pressure is elevated, so we meet with a paradox whic has to be resolved if we wish to attach any significance at all to th hypothetical possibility that the changes in interstitial pressure may be a factor concerned with the movement of these ions

This paradox led us to reinvestigate the effect of ureteral pressure elevation on these functions, and the next series of experiments has to do with the effects of elevated ureteral pressure on the same functions that we have examined with the modification of arterial pressure

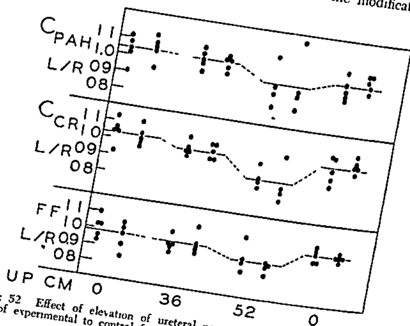


Figure 52 Effect of elevation of ureteral pressure on renal function given as ratios of experimental to control function

In Figure 52 are data from a group of five animals given rapid saline infusion before and during elevation of ureteral pressure in one kidney The ureteral pressure values are given in terms of elevation above the control kidney, in two stages with two urine collection periods at each stage We did this very simply by putting plastic tubing into the ureters, keeping one fixed at a low position and

gradually raising the other in the two stages measuring the difference in the outflow level and using that as the pressure exerted on the ureter and the kidney We did the first stage at 36 cm of elevation and then 52 cm of elevation of the experimental ureteral pressure above the control

I should be happy to give the absolute data for these experiments but I think that the ratios are, perhaps, more significant in terms of controlling the nonspecific factors It can be seen that the first stage of ureteral pressure elevation changes the para-aminohippurate clearance little, if at all The second stage of ureteral pressure elevation does have a slight influence on the para aminohippurate clearance, certainly in three of these dogs

Observe the effects on creatinine clearance taken to measure the filtration rate Here the response is a little bit greater than that of para-aminohippurate, not very marked in the first stage but down to about 88 per cent of control in the second stage

Winton What is the arterial pressure?

Selkurt The arterial pressures were all quite similar in these animals They ran about 130 mm Hg and remained constant throughout the experiment

The filtration fraction shows a small decrease in the first period and a greater one in the second period which indicates that the elevation of ureteral pressure had a greater influence on filtration rate than it did on the para aminohippurate clearance, and raises the question of the mechanism for reduction of filtration rate when ureteral pressure is elevated

Two things are probably involved One is the slight impairment of blood flow, which may be the result of compression of the capillaries by the elevated intrarenal pressure which results when the ureteral pressure is increased In agreement with Dr Winton, I can see no reason why that cannot be the explanation The collecting tubules probably expand and compress the vasculature, perhaps indirectly increasing the renal interstitial pressure This pressure may be transmitted as far as the glomerulus But the fact that the filtration fraction decreases indicates, very possibly, that some additional factor is involved in the reduction in filtration rate, and here too, I favor Dr Winton's explanation that it is possible that the elevated pressure may back up in the lumen of the nephron all the

clearance goes down more than the simultaneous plasma flow

Swann I am interested in that figure of 36, because there is my magical figure of approximately 25 millimeters of mercury Very little effect is seen unless 25 millimeters of mercury is exceeded

Selkurt Yes You can very well guess why we took these figures as our experimental values

Shannon You are prejudiced, Dr Swann

Swann Yes, I am afraid so

Selkurt We were more interested in the possible effects on the electrolyte and water excretion which would result from the increase in ureteral pressure, and those data appear in Figure 53, again as

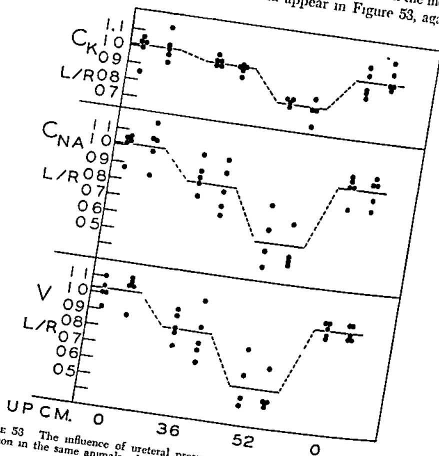


FIGURE 53 The influence of ureteral pressure elevation on electrolyte and water excretion in the same animals which appear in Figure 52

ratios The potassium excretion or clearance is given at the top of the Figure, next, the sodium excretion or clearance, and at the

bottom the urine volumes are shown. Again there is a more marked effect, as measured in percentages, on the excretion of sodium and urine than on the other functions. In absolute terms the excretion of sodium here diminishes by about 160 microequivalents, that is to say, the experimental kidney drops to 160 below the control, and they begin at about 300 to 350 milliequivalents per minute of excretion; thus it falls about half at the highest stage. The effect is felt early. The first pressure elevation produces what is probably a

second stage

stage and is

The interest

ing thing here is that there is a close correspondence between the *alteration in sodium and in urine volume.*

What is the mechanism for diminished excretion under these circumstances? Are we favoring or enhancing the tubular reabsorption of sodium and water by the elevation of the ureteral pressure? That was the first possibility we had to consider. Could it be explained in terms of other factors, such as, for example, a change in load suggested by the changes in filtration rate shown in Table VI? And again, what could the possible influence on these functions be of our hypothetical situation of altering intrarenal pressures?

We believe that the first possibility certainly is not the case. There is no evidence in our series that elevating ureteral pressure in any way directly enhances the reabsorption of sodium and water. We feel that, if anything, it inhibits it. The reason for believing this is shown by such experiments as appear in Figure 54, one of a series of five experiments in which the same experimental procedure was used, except that maximal tubular glucose reabsorption (Tm) was also measured. In this Figure the absolute amount of sodium reabsorbed is indicated (T_{Na}). One observes a decrease in the excretion of sodium, a somewhat lesser but definite change in excretion of potassium, a reduction in glucose Tm, and an absolute reduction in the amount of sodium reabsorbed (T_{Na}).

We may take this as evidence that enhanced reabsorption of sodium does not explain the diminution of excretion. The reduction in glucose Tm poses further questions. I might add that the plasma glucose levels in this animal were always kept at a ratio of load to Tm higher than 1.7, so the fall in glucose Tm came about with a great surplus of glucose in the plasma. The classical interpretation of the decrease in glucose Tm with adequate glucose loading is that nephrons must stop their functioning. If the Tm of glucose falls with the creatinine clearance, then the fall in creatinine clear

Swann I am interested in that figure of 36, because there is my magical figure of approximately 25 millimeters of mercury Very little effect is seen unless 25 millimeters of mercury is exceeded

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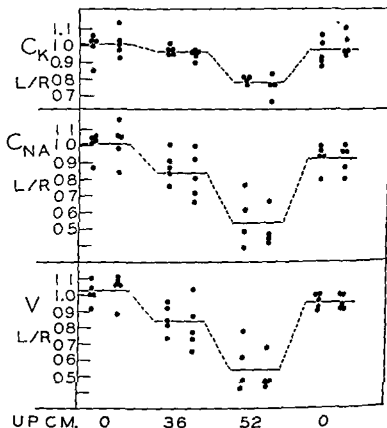


FIGURE 53 The influence of ureteral pressure elevation on electrolyte and water excretion in the same animals which appear in Figure 52

ratios The potassium excretion or clearance is given at the top of the Figure, next, the sodium excretion or clearance, and at the

bottom the urine volumes are shown. Again there is a more marked effect as measured in percentages on the excretion of sodium and urine than on the other functions. In absolute terms the excretion of sodium here diminishes by about 160 microequivalents; that is to say, the experimental kidney drops to 160 below the control and they begin at about 300 to 350 milliequivalents per minute of excretion; thus it falls about half at the highest stage. The effect is felt early. The first pressure elevation produces what is probably a significant effect but the effect is much greater in the second stage. The effect on potassium is quite slight in the first stage and is undoubtedly significant at the higher ureteral pressure. The interesting thing here is that there is a close correspondence between the alteration in sodium and in urine volume.

What is the mechanism for diminished excretion under these circumstances? Are we favoring or enhancing the tubular reabsorption of sodium and water by the elevation of the ureteral pressure? That was the first possibility we had to consider. Could it be explained in terms of other factors, such as, for example, a change in load suggested by the changes in filtration rate shown in Table VI? And again, what could the possible influence on these functions be of our hypothetical situation of altering intrarenal pressures?

We believe that the first possibility certainly is not the case. There is no evidence in our series that elevating ureteral pressure in any way directly enhances the reabsorption of sodium and water. We feel that, if anything, it inhibits it. The reason for believing this is shown by such experiments as appear in Figure 54, one of a series of five experiments in which the same experimental procedure was used except that maximal tubular glucose reabsorption (Tm) was also measured. In this Figure the absolute amount of sodium reabsorbed is indicated (Tx). One observes a decrease in the excretion of sodium, a somewhat lesser but definite change in excretion of potassium, a reduction in glucose Tm, and an absolute reduction in the amount of sodium reabsorbed (Tx).

We may take this as evidence that enhanced reabsorption of sodium does not explain the diminution of excretion. The reduction in glucose Tm poses further questions. I might add that the plasma glucose levels in this animal were always kept at a ratio of load to Tm higher than 1.7, so the fall in glucose Tm came about with a great surplus of glucose in the plasma. The classical interpretation of the decrease in glucose Tm with adequate glucose loading is that nephrons must stop their functioning. If the Tm of glucose falls with the creatinine clearance, then the fall in creati-

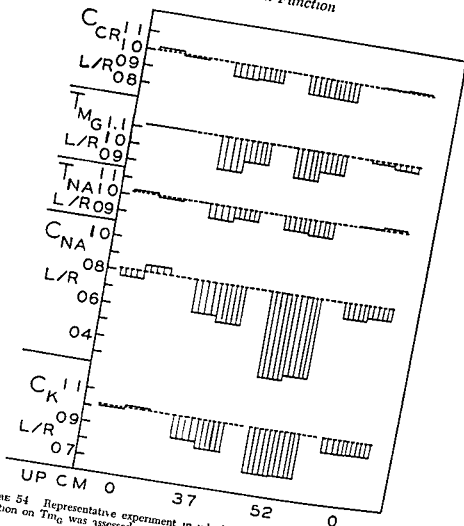


FIGURE 54 Representative experiment in which the influence of ureteral pressure elevation on Tm_G was assessed in addition to functions previously investigated

ance must result from cessation of filtration in certain glomeruli Actually, the Tm_G changes somewhat more than creatinine in this particular experiment However, if we examine the averages for all dogs there is a rather close correspondence of the percentage change in Tm_G and the percentage change in creatinine clearance, which suggests that when ureteral pressure is elevated, the pattern of filtration rate is not altered equally in all nephrons Why or how, I cannot say for certain This should be kept in mind in explaining the decrease in T_{Na}

We were also particularly interested in trying to explain why sodium and water excretion diminishes when venous pressure is increased, and, as you know, some of the earlier ideas on this have been that there is, in fact, an enhanced reabsorption of sodium and water as a result of this experimental modification If we put them both on the same ground, I think we can look for possible common mechanisms and explain both at once thus killing two birds with one stone, so to speak This approach seems logical because it is probable that elevation of venous pressure acts very much like the elevation of ureteral pressure on these functions and probably for similar reasons When the ureteral pressure is elevated it is likely that the expanded collecting tubules compress the veins and produce venous obstruction, and conversely when the venous pressure is elevated, the veins expand and the collecting tubules are compressed with an effect like that of ureteral obstruction

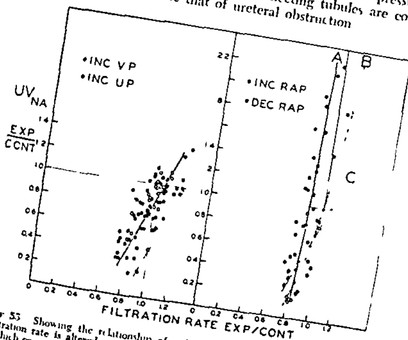


FIGURE 55 Showing the relationship of sodium excretion to filtration rate when the filtration rate is altered with different experimental procedures To the left are data which compare the effects of elevation of renal venous pressure (solid symbols) to ureteral pressure elevation (open symbols) The mean of these data appear to the right as line C Line A mean of data relating sodium excretion to changes in filtration rate when renal arterial perfusion pressure is varied Line B mean trend of data when filtration rate is varied by rapid infusion of isotonic saline solution This curve is derived from the data in Figure 50 (see text)

At the left on Figure 55 are compared the results of the effect of increased ureteral pressure (data in which no glucose was infused) with the effects of venous pressure elevation On the left hand side of the Figure, the excretion of sodium, UV_{Na}, expressed as a ratio of experimental to control, is related to the percentage change in filtration rate The lines indicate 100 per cent The solid circles are data, which were published early this year(6), derived from twelve dogs in which venous pressure was elevated All stages of control, three stages of venous pressure elevation and recovery, are included, accounting for some of the scatter in the data The solid diagonal line represents the arithmetic mean of these experiments, showing the relationship of excretion of sodium to filtration rate and the dotted lines are plus or minus twice the standard deviations

Our reason for believing that the ureteral pressure elevation factors are exactly the same as the venous pressure elevation is the fact that these points (open circles) are in the same position Their mean trend follows the mean line and their scatter is within the range of the data obtained in a study of the effects of venous pressure elevation We see therefore, that there is some sort of relationship between the excretion of sodium and the reduction in glomerular filtration rate in both cases, and that the underlying phenomena seem to suggest that exactly the same amount is absorbed with venous pressure elevation as with ureteral pressure elevation when filtration rate is decreased to a corresponding degree

Is this decrease in sodium excretion and urine volume the result of the decrease in filtration rate, or is it coincidental? If it is a matter of changing sodium load do other factors modify this situation?

It seems to me that the first question can be answered if we change filtration rate in some other way and determine if the change in sodium excretion is the same But for the moment, we shall digress to another series of data which supply the basis for our thinking on this In this study (Figure 56) we attempted to assess the effect of altering filtration rate on sodium excretion by a different mechanism Here again — and I wish there were a better way — rapid infusion of isotonic saline was used at the rate of about 20 ml per minute to elevate filtration rate rapidly These plots are the average trends of seven experiments All measurements were made within a period of about an hour The kidneys were weighed, all values were referred to unit weight and then averaged to give this curve, so that we could relate the changes in tubular

sodium reabsorption (labeled here T_{Na}) and sodium excretion (UV_{Na}) with the change in filtration rate. These data are corrected for one kidney only.

Our animals had a range of filtration rate from about 25 to 40 ml per minute per kidney and it can be seen that as the filtration rate increases with rapid saline infusion there is a corresponding increase in

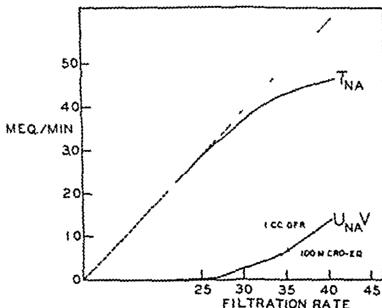


FIGURE 56. Showing the relationship of tubular sodium reabsorption and urinary excretion when filtration rate is rapidly increased by isotonic saline infusion. The curves are averages derived from seven experiments, and data are given for one kidney only.

crease in excretion and a corresponding partial inhibition of reabsorption. We are now dealing with a classical phenomenon which suggests that the tubular mechanisms can be in part saturated under a load. We chose isotonic saline because we wanted to keep nonspecific alterations to a minimum. *I might also add that these* experiments were selected because there were no great changes in arterial pressure and very few if any changes in venous pressure. We tried to screen the animals from the standpoint of other factors which might be concerned, such as constancy of plasma sodium, and we tried to relate as specifically as possible the excretion of sodium with changes in filtration rate. The thing that struck us as remarkable, and we had not been conscious of it before, is

that, at least in this group of data, the excretion of sodium can be changed by *100 microequivalents by changing the filtration rate* only 1 ml per minute That is a fundamental thing to keep in mind in analysis of the data presented earlier, because *it suggests that there is some inherent trait of the sodium reabsorption mechanism* whereby the excretion can be, both absolutely and with reference to percentages, changed greatly with minor modifications in filtration rate

Returning now to Figure 55, let us try to reach some final conclusion The trend of the change in excretion of sodium with changes in filtration rate, occasioned by venous pressure and ureteral pressure elevation, appears as line C to the right Line B is the curve derived from the data just given, in terms of percentile changes, by taking the mean of all the control filtration rates of all the mass data (35 ml per min per kidney), and setting this at 1, and then taking ratios of filtration rate above and below Ratios of sodium excretion above and below the mean excretion at 35 ml per min filtration rate supply a curve which is, in a sense, analogous to the curves supplied for C and A

Line A is the combined data of the earlier animals in which the perfusion pressure was reduced in five animals and elevated in the other five We have plotted the relationship in exactly the same way, the change in excretion relative to filtration rate We started slightly below 1 because our experimental kidney presented a filtration rate of only about 90 per cent of the control The solid symbols are the points of increasing pressure and the open circles are the points with decreasing pressure

The general correspondence of these curves and the relationship of filtration rate to excretion (obtained in three different ways) suggested certain underlying things in common It can be seen that the line C for the changes of sodium excretion in ureteral elevation is close to the line B, suggesting that a fundamentally similar mechanism probably operates in both cases And we may go on to say that we believe the decrease in excretion of sodium and urine volume is fundamentally the result of decreasing sodium load by the very minor changes in filtration rate which result from that procedure

Line A probably has as its basis a somewhat similar phenomenon, but the very steepness of it suggests two things about the variation of arterial perfusion pressure One possibility is that if these changes are solely the result of changes in load, then the excretion is extremely sensitive to changes in filtration rate The other possibility

is that the change in load is one factor but that there is another factor which accelerates or favors the change in excretion along with the change in filtration rate

Now can we finally decide as to the possibilities? I would think that we could simply close the discussion by saying that all observed changes in excretion of sodium and urine volume whether we vary arterial pressure or ureteral pressure or venous pressure are due to the same thing some fundamental inherent characteristic of the loading reabsorptive processes in the tubular cell But I am going to be stubborn enough to suggest that there are probably other factors involved and the main reason for saying this is that there is a rather noticeable difference in the slopes of lines A and C With elevation of renal arterial pressure the excretion of sodium is aided and retention is favored when it is lowered When the venous pressure and the ureteral pressure are increased the more gradual slope of the curve indicates that if anything there may be a degree of interference with the reabsorption of sodium and water even though the net effect is a reduction in excretion

The only possibility that may explain these differences concerns the role of the intrarenal pressure We know that the intrarenal pressure increases when the venous pressure is elevated and when the ureteral pressure is elevated It does so in the face of reduced loads *Therefore if the increased intrarenal pressure as an isolated* factor would be one which inhibited the movement of sodium and water from the tubular lumen to the capillary blood then these curves should be exactly identical But there is one important and significant difference When perfusion pressure is increased by aid of the pump intrarenal pressure is probably increased Filtration rate if anything increases so that any possible factor of encroachment on

the intra

out and

elevation of intrarenal pressure as it acts on the movement of ions and water from the lumen to the capillary may be felt maximally On the other hand when the intrarenal pressure is increased with ureteral or venous pressure elevation at the same time blood flow is impaired the glomeruli are compressed and the sodium load is reduced so that two opposing factors operate which in a sense might cancel each other out to a degree

With those speculations I shall close this discussion and leave it to be decided if there are factors which might offer a better explanation than the possible role of the intrarenal pressure which

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The only possibility that may explain these differences concerns the role of the intrarenal pressure We know that the intrarenal pressure increases when the venous pressure is elevated and when the ureteral pressure is elevated It does so in the face of reduced loads Therefore, if the increased intrarenal pressure as an isolated factor, would be one which inhibited the movement of sodium and water from the tubular lumen to the capillary blood, then these curves should be exactly identical But there is one important and significant difference When perfusion pressure is increased by aid of the pump, intrarenal pressure is probably increased Filtration rate if anything, increases, so that any possible factor of encroach-

elevation of intrarenal pressure as it acts on the movement of ions and water from the lumen to the capillary may be felt maximally On the other hand when the intrarenal pressure is increased with ureteral or venous pressure elevation, at the same time blood flow is impaired, the glomeruli are compressed, and the sodium load is reduced so that two opposing factors operate which, in a sense might cancel each other out to a degree

With those speculations, I shall close this discussion and leave it to be decided if there are factors which might offer a better explanation than the possible role of the intrarenal pressure, which

becomes a biophysical problem More questions are raised than are answered, for example, the magnitude of the pressure changes in the kidney and their possible relationship to the movement of ions in water In terms of dissociation constants, it hardly seems possible that the relatively small magnitude of pressure changes here would be enough to alter specifically the movement of sodium However, they are well within the range of hydrostatic influences on movement of water Perhaps the answer lies there, but we cannot arrive at a final conclusion because sodium and water always move together in the same proportion

Berliner Do you know whether the slopes of lines A, B, and C in Figure 55 are significantly different?

Selkurt That is a question which I shall answer in this way I do not believe, because of the scatter of the data, that significance could be ascribed in terms of statistical analysis It is obvious that these points of the two groups will overlap to a considerable degree It is possible that data determining these lines (A compared to C) are significantly different as compared to ones which derive line B, but I would not bet on it It would be very difficult to prove, and I am just hinging my argument on the significance of mean trends of large groups of animals If you wish to dismiss that as not being a good way of reasoning, why I won't argue too loudly because you are probably right It hardly justifies speculation perhaps

Berliner There is one disturbing thing about the group of animals in which arterial pressures were varied, that is, they are starting out with an experimental to control ratio around 0 8 instead of approximately 1

Selkurt No it is actually 0 88

Berliner They are significantly different from the other animals

Selkurt That is true We are doing the best we can, with a lot of plumbing between the kidney and the blood supply, and it cannot be brought up to 1 If you want to shift line A over, as I said, from 9 to 1 and bring the lines together, the slopes would still be different

Berliner Yes, there are many technical difficulties What I am aiming at is that since something has already happened to these kidneys to make them different from the others, they might not respond to superimposed changes in the same way

Selkurt That is a good point The only thing I can suggest is that when the experiment is done, as we did it by constricting the aorta between the kidneys, there is the same change that is obtained with the plumbing in place by compressing the Tygon tubing We

could not of course go the other way in those experiments so I cannot answer that point but it is a valid criticism

Dock Is there much difference in function between a dog's left and right kidney?

Selkurt They are usually very close as you can see from the data on ureteral pressure The reduction in ratios has something to do with the running of blood through the plumbing What it is I don't know It might be a purely hemodynamic thing in terms of the fact that a pressure drop results across that circuit With a pump that is obviated because the pump pressure going into the artery is maintained the same or slightly higher than the control pressure so that particular argument is eliminated

Swann In confirmation of your theory rapid infusion of saline elevates the interstitial pressure greatly It raises it in fact to about 60 mm Hg

Selkurt I certainly thought of that and if that is true then all three groups fall into the same category Intrarenal pressure is elevated along with increasing load and the intrarenal pressure may make a factor which adds to the excretion of sodium with infusion of isotonic saline

Swann Another fact about interstitial pressure which may be of interest to you in subsequent work is that interstitial pressure is inversely related to the protein osmotic pressure It is low when protein osmotic pressure is high and high when osmotic pressure is low The same paired observations have been made on the eye by Duke Elder(7)

Selkurt I should like to comment on the question of whether the filtration rate in clinical conditions is a factor concerned with sodium excretion Those who say that filtration rate does not change may be ignoring changes of as much as 5 or 10 ml per minute which may be very important

Shannon May I make a comment on that Dr Selkurt? Our measurements are not sharp enough to differentiate between two situations wherein there are small percentile differences in glomerular filtration rate even though the differences may have marked functional significance This is clearly brought out by some of the *data reported here last year by Dr White from St Louis(8)*

Selkurt That also applies to Blake's observation that changes [illegible]

detect significant changes They probably are not bee[illegible]

recognize chemical errors of 3 or 4 per cent, and so on

Winton There is this question of pulse pressure I should like to remark, in connection with Hooker's experiments(3) in which kidneys were perfused with Ringer's solution, that there probably was very high intrarenal pressure, and, as far as I can remember, the indication was that his data were open to criticism for two reasons one, the diastolic phase of the perfusion pressure dropped below the intrarenal pressure and therefore the flow probably ceased altogether, and, second, there were inadequate controls of mean pressure I think these data really should be forgotten

Selkurt Well, Gesell s data(4) are a little more acceptable, don't you agree?

Winton Yes, but a great many people have investigated this, including yourself and have come to the conclusion that if you keep well above the intrarenal pressure, you do not show much effect of pulse pressure on renal blood flow, so long as you control mean pressures at exactly the same value I wanted to ask how you had done that because it is not easy to decide what the fair mean of a pulsatile pressure really is The way I have controlled it is by having a parallel perfusion through a glass tube and adjusting pulsatile and smoothed blood pressures so that the blood flow through the glass tube remains the same If it does not change, I assume I have had the same pressure whether or not there was pulsation If you try to take the mean of a pressure known not to be sinusoidal, a heart beat or something of that kind, you are not going to get anything like the value you really need for this purpose

Selkurt In other words, how did we get the mean pressure from our data?

Winton Yes

Selkurt We had a mercury manometer hooked up that we switched back and forth and one can take the optical record and integrate it, getting the mean pressure from the curve itself

Winton It can be done that way, it is quite true but I wonder whether you have, in fact, done it

Selkurt We checked it both ways, and, within the minor fluctuations that go on, it is reasonably accurate Small changes with respiration and things like that have to be taken into account

Mudge Do you think you would reach different conclusions if you plotted your data comparing the function of each kidney against what it was at the beginning of the experiment?

Selkurt The reason why that leads to difficulty is that these are anesthetized and heparinized dogs, and, with an external circuit

in, there is always a gradual downward trend in these functions

Mudge That is just the point I am getting at When a clearance is measured in a normal dog, the rates of sodium and water excretion tend to fall if the filtration rate decreases The slopes of the curves of your mass plots might be quite different if the experimental periods were compared to the so called normal state I wonder to what extent the difference between your curves might be due to the arbitrary way in which the data have been calculated

Pitts Experiments performed by Dr Thompson(10) in my laboratory bear on this problem Although these experiments were performed under anesthesia, opening of the abdomen was avoided by the use of a balloon catheter which could be introduced through either the femoral or common carotid artery Figure 57 illustrates in lateral view the radiographic picture of the balloon *in situ* inflated with diodrast It was introduced through one femoral artery



FIGURE 57 Lateral view of the thorax and abdomen of a dog with the balloon catheter [illegible]

and positioned fluoroscopically above the origins of the renal arteries The opposite femoral artery was cannulated for the estimation of renal arterial pressure By inflation of the balloon, renal arterial pressure and hence filtration rate could be reduced to any desired level

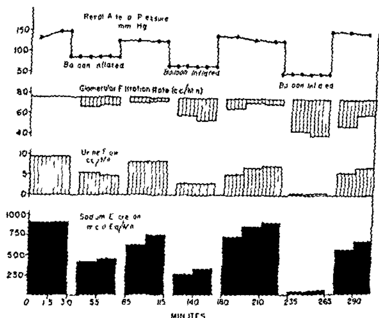


FIGURE 58 The effects of lowering the renal arterial pressure on glomerular filtration rate urine flow and rate of sodium excretion in an adrenalectomized dog Reprinted by permission from Thompson D D, and Pitts R F *Am J Physiol* 168, *490 (1952)*

Figure 58 shows graphically the data obtained in one such experiment performed on an adrenalectomized dog When the balloon was inflated, and the pressure reduced from around 150 to 90 mm of mercury, glomerular filtration rate, urine flow, and sodium excretion all decreased However, sodium excretion was reduced to a greater extent than were the other two variables When renal arterial pressure was allowed to return to its control level, all variables approached control values With greater inflation of the balloon and greater reduction in arterial pressure in the two succeeding instances, the effects were more marked During the final period of inflation, in which glomerular filtration rate was reduced roughly by half urine flow and sodium excretion were reduced to less than one tenth their control values

Pappenheimer During saline diuresis?

Pitts Yes, saline was infused throughout all experiments However, in order to avoid the accumulation of an increasingly positive sodium and water load during periods of reduced filtration rate, the rate of infusion was cut to equal the urine flow

Figure 59 is a mass plot of all data relating sodium excretion to glomerular filtration rate It includes a series of experiments on

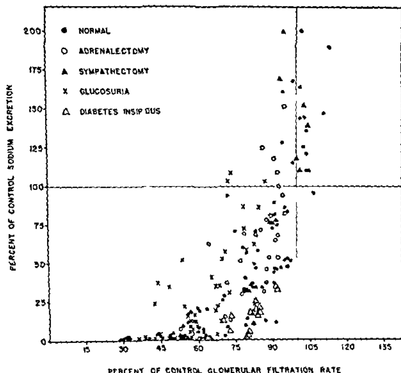


FIGURE 59 The relationship between rate of sodium excretion and glomerular

normal animals experiments on two adrenalectomized animals, and on two animals with diabetes insipidus induced by stalk section two animals with unilateral splanchnicectomy, and, finally, the experiments on a group of animals infused with glucose for the measurement of glucose Tm Along the ordinate is plotted sodium

excretion in per cent of control and along the abscissa is plotted filtration rate likewise in per cent of control It is evident that as glomerular filtration rate is reduced sodium excretion drops off rapidly to reach very low values at filtration rates below 50 per cent of normal

Figure 60 illustrates these same data in a plot relating per cent of filtered sodium absorbed to glomerular filtration rate It is evident that sodium absorption becomes nearly complete when filtration rate is reduced below half its control value

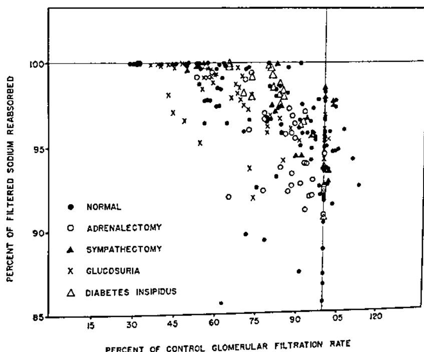


FIGURE 60 The relationship between rate of sodium reabsorption and glomerular filtration rate both expressed in per cent of control values from the series of experiments summarized in Figure 59 Reprinted by permission from Thompson D D and Pitts R F *Am J Physiol* **168**, *490 (1952)*

In Figure 61 urine flow is related to glomerular filtration rate For the most part urine flow drops off sharply to reach very low values as filtration rate is reduced to half its normal value However the urine flows in the glucose infusion experiments are distinctly out of line a consequence no doubt of the osmotic effects of the urinary load of glucose Despite the higher urine flows in the glucose experiments sodium excretion dropped with filtration rate much as

it did under other conditions In these experiments, glucose Tm was relatively unaffected by halving filtration rate We conclude, therefore, *that a reduction in the quantity of filtrate formed in each* glomerulus is associated with excessive reabsorption of sodium and water That this excessive reabsorption is due primarily to the change in the quantity of filtrate formed and not dependent on hormones or nervous influences is indicated by the similarity of results obtained in adrenalectomized diabetes insipidus, and sympathectomized animals

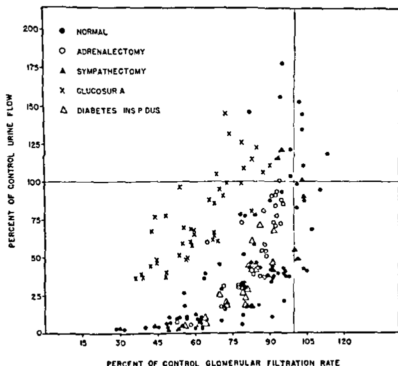


FIGURE 61 The relationship between rate of urine flow and glomerular filtration rate both expressed in per cent of control values from the series of experiments summarized in Figure 59 Reprinted by permission from Thompson D D and Pitts R F *Am J Physiol* 168, 490 (1952)

Selkurt I think the dominant thing is the change of filtration rate *But what is your feeling on that Dr Pitts?* You obtained about the same spread and a very rapid change in sodium excretion as the filtration rate was decreased

Pitts I think I would interpret it as a consequence of varying the sodium and water load

Selkurt What goes on at the level of the cells themselves?

Pitts If you want to put it in specific terms, to which Dr Oliver may rightly object, I would say that the proximal absorption of sodium is, in percentages, increased as one reduces the load by lowering filtration rate Distal absorption, if a Tm exists as Dr Homer Smith believes would continue at a constant rate until the load delivered to this segment falls below its absorptive capacity At a filtration rate half the normal, combined proximal and distal absorption removes nearly all of the sodium from the urine My basic thesis is that there is no such thing as an over all Tm for sodium, at least nothing which bears much resemblance to the proximal Tm for glucose The sodium system is of an entirely different type, and I would follow along, I think, with Dr Mudge's views(11) and with those of Drs Wesson and Anslow(12), that when one deals with sodium, one is not dealing with Tm in the usual sense of limited or restricted absorptive capacity

Selkurt How do you explain the results when one pushes hypertonic sodium chloride into dogs and obtains a splay when relating the amount reabsorbed to load? This often shows a remarkable degree of flattening in terms of total reabsorption I recognize the dilemma that might arise in terms of possible changes of filtration but, nevertheless those are the facts I might add that it is colored by the possibility that hormonal imbalances can be rapidly initiated with the hypertonic salt, but the fact remains that when hypertonic saline is rapidly infused into the dog, the excretion increases greatly, and the calculated reabsorption flattens off so that it frequently looks like the glucose Tm

Mudge But when excretion is increased by infusing saline isn't it true that reabsorption is likewise increased?

Berliner When there is no appreciable change in filtration rate, reabsorption seems to level off pretty well, but when the filtration rate changes, it does not

Pitts That doesn t mean Tm, though, in the usual sense in which it is applied to glucose, because there Tm is independent of filtration rate Here, there is a Tm which is distinctly dependent on filtration, which to me is not Tm

Shannon To my mind, under certain general conditions there is a maximum rate of tubular transfer which is reproducible This is commonly called the Tm for a substance The data on sodium yield figures which are not reproducible

Winton Did you, in your rapid Ringer infusion experiments, get a large reduction of reabsorption of water?

Selkurt No, I wouldn't call it a large reduction

Winton We did in our dogs, a very large reduction of reabsorption of water and very small changes of glomerular filtration rate, i e, creatinine clearance, and when we neutralized it by raising the water pressure, we still got the large diuresis

Selkurt I have also observed a diuresis with saline without any remarkable change in filtration rate Something is going on in the tubular cells The reabsorptive capacity is changing

Fremont-Smith Doesn't it make a saline diuresis comparable to a water diuresis?

Shannon Dr Fremont-Smith, we worked with dogs, using saline and I don't think we ever saw a diuresis in such a preparation without an increase in filtration rate

Pitts I think that is probably true of the dog, but as I remember some experiments of Ladd(13) that came from Homer Smith's laboratory, in man they obtained quite phenomenal diuresis on the administration of saline if the individual had been hydrated by the administration of large water loads some twelve hours before

Berliner They got a water diuresis They gave saline but got a water diuresis

Fremont-Smith That depends on how you use the term "water diuresis" You call it water diuresis because there was no change in filtration

Berliner No, I call it a water diuresis because the osmotic pressure of the urine drops and the urine becomes very dilute

Fremont-Smith Then you have to decide whether you are calling a thing water diuresis because it is induced by drinking water or because of the quality of the urine They are not exactly the same, and the implication is that this is dismissed as water diuresis because the urine looked like that during a water diuresis

Berliner What I had in mind when I said that they had a water diuresis was that what came out was water, not what they took in

Winton In the isolated kidney, saline diuresis is due to an increase in glomerular filtration without much change in tubular activity In the anesthetized dog saline diuresis is mainly due to less reabsorption of water in the tubules(14)

Selkurt That may be the answer to what is going on here because our data were on anesthetized animals and apparently what Dr Shannon is talking about is unanesthetized animals

Shannon The conventional anesthetics are commonly characterized by a lowering of the glomerular filtration rate

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THE USE OF THE ARTIFICIAL KIDNEY

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IN DISCUSSING THE use of the artificial kidney it is wise to bear in mind the fact that the human kidney has been functioning for a good deal longer and with a good deal more precision than any artificial kidney The data which I am going to discuss this morning are necessarily scanty but some analogies may be drawn and I think perhaps some idea can be given of the present role of the artificial kidney as a therapeutic and investigative tool

It might be of interest to introduce the subject by making some comparisons between the filtering or dialyzing membrane which we use and the human glomerular capillary membrane (I am indebted to Dr Pappenheimer for the data on the capillaries) In employing the term filtration" as I do I realize that I am on thin ice In increasing the hydrostatic gradient across the cellophane membrane from the inside out we feel we are counteracting as it were the osmotic pull of the blood within the cellophane membrane and by raising that hydrostatic pressure further may actually increase water flow to the point where negative balances for water may be achieved Such an interpretation of "filtration" is limited and is fraught with difficulty but it does seem to be a clinical fact Whether one subscribes to the pore theory or to the gel theory I think the relation of the capillary to the cellophane membrane is of interest and can be interpreted in terms of Dr Pappenheimer s results

The cellophane membrane which is used in most of the apparatus today is actually a commercial grade of prepared cellulose sheet which is manufactured primarily for packing purposes No attempt has been made to control pore size or to produce a film which will meet specific requirements as a dialyzing membrane As it is used generally the plain non moistureproof variety is an unregenerated sheet or a seamless tube which is prepared with glycerol or other softening agent in aqueous solution This is necessary to prevent the collapse of the cellulose structure This cellulose or cellophane membrane comes to us direct from the manufacturer of sausage casing and is exactly the same sort of thing that the packing people use to prepare their frankfurters There is no difference except that we treat it with water to make it a more efficient agent of transfer

For *in vivo* work, this membrane must be washed and soaked so that the softening agent, in this case glycerol, which is water-soluble, can be removed. As it is prepared for clinical use, of course, it must be boiled to sterilize it, and the cellophane absorbs large quantities of the water, becoming grossly swollen. In this swollen condition, the general permeability is of an entirely different order of magnitude from that of the dry, or the glycerol-prepared sheet. In this distended condition, it is our feeling, and that of others, that it contains capillary channels of submicroscopic size.

The pore radius in the swollen state has been estimated, by workers using it for other purposes, at anywhere from 20 to 95 Ångstrom Units, depending upon the grade of cellophane used. People who have used it in physical chemistry and in bacteriology feel that the grade of cellophane now has a different order of pore size and fractional pore area than it used to have, and we have some evidence for that although in the last two years or so the membranes which we have received have been reasonably consistent. By actual measurement with the method of Bauer and Hughes(1) the mean pore radius of the cellophane in our apparatus as it is prepared for dialysis, that is, as it is swollen with water, is about 30 Ångstrom Units. This we might compare with the data given for the capillary by Dr. Pappenheimer. Cellophane with a pore radius of 30 Ångstrom Units is roughly comparable to the muscle capillary, if one interprets these data on the uniform pore theory. For the rectangular slits, it is perhaps a little less. The pore radius in the glomerular capillary is probably slightly larger, but in any case it is of the same order of magnitude.

Collodion may vary greatly but the characteristics of collodion membranes can be controlled with more accuracy. Collodion membranes have been prepared with pore radii ranging from 12 to 200 Ångstrom Units simply by changing the proportion of solvent and the time for gelling and evaporation. We have attempted to make graded collodion membranes and we have had some success in controlling the pore size but we have been unable to produce a membrane with a reasonable thickness. The thickness of cellophane in the swollen state is extremely important since the seamless tubing which we are using — and this is true for the #300 gauge sheets which are used in other types of apparatus — is about 80 microns. In comparison with the capillary wall thickness of 1 micron or less, this is very large, and it is a factor of extreme importance in controlling the rate of transfer across the membrane.

particularly when that rate varies inversely with the square of the pore length in this case the wall thickness It is possible to control pore size in cellophane to some extent Pore radius may be in creased by treatment with zinc chloride and sodium hydroxide but for clinical work the treatment of 108 feet of seamless tubing by zinc chloride and sodium hydroxide and the necessary washing is a pretty difficult job As far as I know these two methods are the only satisfactory ways of increasing the pore radius and unfor tunately the wall thickness is also increased

The fractional pore area that is the actual space available in the membrane for transfer in the cellophane according to our calculations is about 30 to 45 per cent In the muscle capillary as Dr Pappenheimer pointed out the data may be interpreted as evidence for a fractional pore area of about 0 0001 per cent and in the glomerulus 0 01 per cent

The total area of the human glomerular capillaries has been variously estimated and two such estimations are 7600 and 15 000 square centimeters The dialyzing area or at least the area exposed for dialysis of the Kolff apparatus is about 22 000 square centimeters of cellophane I think this is about the largest area that is in use today This value is as low as 6500 in other types of apparatus

In the collodion membrane a mean pore radius of from 100 to 120 Ångstrom Units makes it about 95 to 99 per cent permeable to serum albumin It must therefore be apparent that cellophane with its mean pore radius of about 30 will readily retain albumin as indeed it does As a matter of fact with the Kolff dialyzer we have not been able to demonstrate any extraction of inulin either on single passages or on repeated circulations of the 500 ml volume with a flow of about 200 milliliters per minute over a period of three hours This is interesting in view of the fact that the effective diffusion diameter for inulin is believed to be about 30 Ångstrom Units

Pappenheimer I should like to point out that inulin will go readily through cellophane if there is a suitable apparatus for measuring it In cellophane there may be a distribution of pore sizes unlike collodion which is relatively isoporous The fact that inulin does go through cellophane membranes can be readily shown

Merrill I am sure it must in the laboratory I am simply pointing out that as used at the clinical level we have been unable to demonstrate any decrease in inulin concentration on single passage through the Kolff dialyzer or on three hour recirculation experi ments

Two functions may be served by the various types of artificial kidneys which are in use at present When the cellophane membrane is impermeable to colloidal solids but passes water and crystalloids, we have dialysis As to filtration, some discussion has already occurred on this subject Nevertheless, the point is that if an apparatus is used in which no attempt is made to increase the hydrostatic gradient from inside out, this is a dialyzer, but if the membrane is supported and the pressure inside is increased or negative pressure if that is a good term is created on the outside by evacuating spaces and supporting the membrane, this is a filter That is the sense in which the apparatus has been used clinically

It should be emphasized that the problem of mechanical design, which has been stressed tremendously in the literature on the artificial kidney perhaps may not be as important as the modification of the membrane particularly because of the important factor of wall thickness I think we have reached the point now where the improvements in mechanical design have gone about as far as they need to in terms of efficacy, and that it might well be possible, by modifying the membrane, to get a dialyzer, or an ultrafilter, which will be equally as effective but a good bit smaller But I do not think that it is possible to do this at the present time by design, and perhaps I can point out why a little later

Some of the other considerations to be kept in mind in the discussion of the artificial kidney are the possible therapeutic implications We know for instance, that the pore radius of the membrane is reasonably fixed if we could control this, if we could effect the transfer more easily of larger molecules, perhaps selectively, we might be able to learn more about the problem of uremia At this time, I am sure that from a clinical standpoint, removal of some of the large amino acids is just not possible at a rapid rate, although some of them do go across But by changing the pore size we may be able to effect more rapid transfer of larger molecules

Of course, the implications of change in wall thickness in terms of decreasing the size of the apparatus itself are obvious

So much for the theoretical considerations I should like to illustrate some of the types of apparatus which are in use today, and then discuss their implications what they do, and particularly what they do not do Figure 62 is an illustration of the so called Kolff apparatus in which blood of the patient or animal is led through a rotating coupling by means of a cannula placed in the artery or by means of a catheter in the inferior vena cava from which blood can be pumped The blood comes in, of course, by

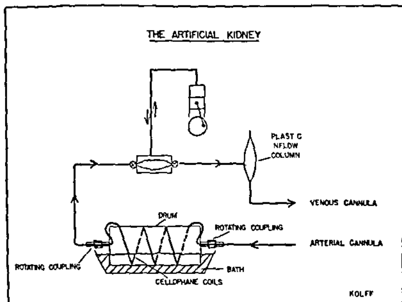


FIGURE 62

arterial pressure However that does not mean that arterial pressure propels the blood through this apparatus for as long as blood reaches the level of the axle the rotation of the drum passes the blood through What actually happens is that this drum acts as a sort of Archimedean screw and a quantity of blood falls down by gravity to the bottom of one of the loops then an increasingly advanced portion of the cellophane is presented to that blood with the clockwise rotation of the drum and the blood is thus advanced In so doing it passes through the bath fluid and transfer of substances occurs across the membrane It is then passed through a rotating coupling again the stationary half of the rotating coupling being connected to the apparatus and the other half rotating with the drum the two having a continuous lumen opposed under spring tension so that no leaks can occur

Then by means of a pump which is actually a plastic bag with one way plastic valves the blood is pumped into a plastic inflow column and fed either under positive pressure or by opening an air vent into the venous side This is a continuous process from artery to vein The bath is maintained at a constant temperature by a thermoswitch and heating units in the bottom of the pan Figure 63 is a photograph of the apparatus The bath is raised

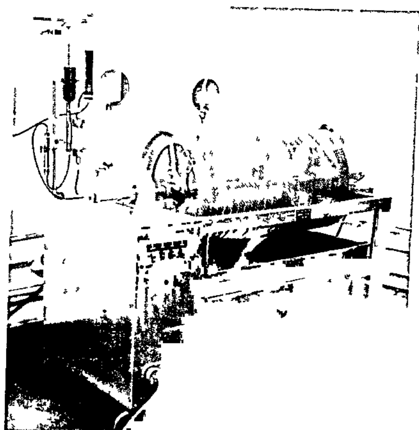


FIGURE 63

during operation so that it covers about 40 per cent of the circumference of the drum The pH of the bath is regulated by CO which sets up a buffer system with the bicarbonate in the bath

There are other types of apparatus Figure 64 is the type devised by Dr Alwall(2) of Sweden and by Dr Murray(3) independently, in Canada In essence this consists of seamless cellophane tubing wrapped in a spiral fashion around a screen with another screen on the outside to give opposing or supporting pressure The Figure shows the two screens with the cellophane membrane interposed between them The blood is pumped by any one of a number of pumps into the tubing and the bath fluid forced in countercurrent flow about the tubing In practice one two or three of these units [illegible] the flow can be from [illegible] paratus with the supported membrane and the pump blood can be pumped through

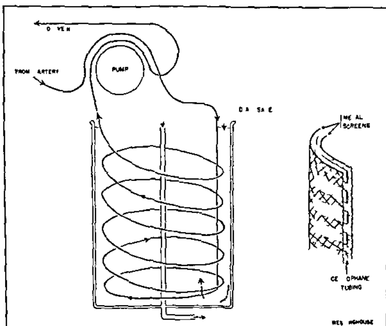


FIGURE 64

under pressures of as much as 200 milligrams of mercury so that filtration occurs The Kolff apparatus on the other hand obviously has no hydrostatic or pressure gradient across the membrane and so is a true dialyzer

Figure 65 is a photograph of the apparatus Each one of the three units consists of two screens with the cellophane tubing interposed between them The pump flowmeter and clot filter may be seen It is possible in addition to pumping blood here on pressure to fill this apparatus completely with water seal it and by dropping a tube at a length from the bottom of the pan again to create a hydrostatic gradient

The third type of artificial kidney – there are others but in general they bear some resemblance to these three prototypes – is that devised by Drs Skeggs and Leonards(4) of Cleveland The one shown in Figure 66 designed by Dr W P Murphy Jr consists of flat sheets of cellophane placed between grooved pads The blood flows within these two sheets A spacer bar is placed so that the sheets will not be compressed by the pads The pad is flipped down and clamped in place The bath fluid flows in one direction

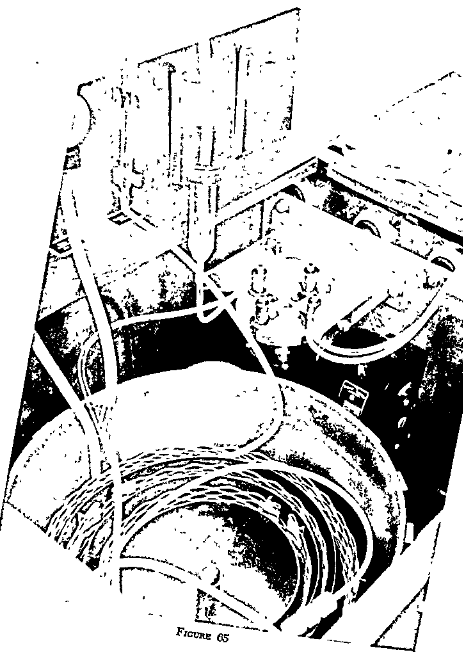


FIGURE 65

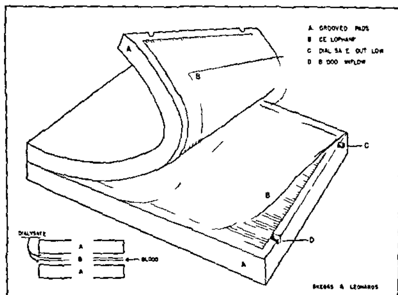


FIGURE 66

on either side of the cellophane membrane and the blood flows through the cellophane membrane One or two or three of these units may be connected in parallel to give increased dialyzing and filtering area

Figure 67 is a model which we have used only with dogs to date of that same type of apparatus slightly modified The cellophane sheet flips down and is clamped in place with a rubber "O ring making a watertight seal The blood flows between the two sheets and out the end and the bath fluid comes in one side above and below the cellophane in a countercurrent flow and is removed at the other side

There is a type of apparatus in which seamless tubing is placed between supports one devised by Dr B D Rosenak and another by Dr W J Kolff but they are essentially the same except that they employ seamless tubing instead of flat sheets Both these apparatus of course are capable of filtering as well as dialyzing

Some of the problems that arise in the construction of an apparatus which will be an effective and safe filter or dialyzer are these First of all an apparatus such as this must be constructed so as to avoid laminar flow in which the flow tends to be more rapid in the center and less rapid toward the periphery so that the

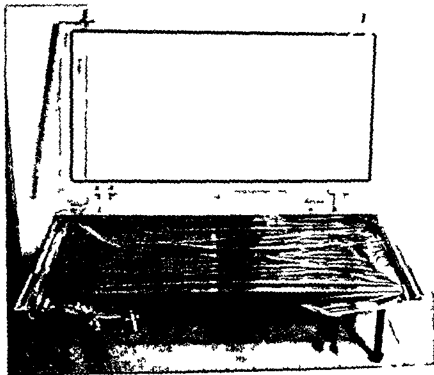


FIGURE 67

whole membrane is not being utilized in terms of exchange Pen

effective apparatus one must have uniform distribu- [illegible] tion of the blood flowing through so that all parts of the blood column are exposed to the surface for exchange as many times as possible That is rather difficult to do *in such an* apparatus and I think Dr Skeggs has approached the problem extremely well

In addition to agitation of the blood the bath itself must be agitated or refreshed It is obvious that if there is a stagnant layer monomolecular or otherwise of bath fluid *on the other side* of this membrane it will soon be *saturated* with transfer of solid and thus will decrease the diffusion gradient As Dr Pappenheimer pointed out the rate of transfer will *rapidly decrease so that it is necessary* to refresh the bath fluid constantly *in order to present a fresh and* solute free bath to the *outside of the* cellophane membrane This has been accomplished in a number of ways first of all by the so called countercurrent flow The Kolff apparatus (Figure 62) is

extremely effective in both these respects because of the nature of the propulsive mechanism What happens is that, as the drum rotates, the loop of cellophane is brought down and actually scrubbed by being immersed in the bath fluid, so that any molecular layer which is saturated with solute at this point is washed free from each loop every time the drum enters the bath Moreover, the movement of the blood in the cellophane loops is not laminar

Oliver What is the rate of revolution?

Merrill About 26 times per minute

Another problem is that of the filter versus the pure dialyzer It is obvious that when a membrane is supported as it must be since the cellophane is very distensible under pressure, the area which is effectively exposed to the bath is decreased In addition, the cellophane bulges through the interstices of even the best-made supporting structure, increasing the volume and causing stasis The effective flow is reduced and may occur with increased pressure Clinically, I feel and I think Dr Pappenheimer believes, that as the hydrostatic head is increased the rate of filtration is increased and dialysis may actually decrease Certainly it has been the experience of anyone who has tried to combine a filter and a dialyzer that when one attempts to filter at 250 millimeters of mercury and attempts to pass blood through such an apparatus at 250 millimeters of mercury, the rate of transfer of solute is apparently impeded The best example of that, perhaps, is the pure filter, in which no water is placed on the other side so that no dialysis occurs This is an extremely ineffective apparatus for the removal of metabolites and has been used only in connection with the removal of large volumes of fluid, thus, in a sense the best filter is the worst dialyzer For these reasons, one must define what he is attempting to accomplish before an apparatus can be constructed for the purpose

Some of the kinetics of the artificial kidney are of interest In the first place, we would like to know what rate of flow through the apparatus is optimum It is apparent that the longer the blood stays in contact across the cellophane membrane with the bath or rinsing fluid, the better the chance of increasing extraction or removal of solute, and that if it is pushed through at a rapid rate the extraction is decreased In Figure 68 we have plotted extraction as the solid line, that is the percentage extraction in one passage through the Kolff dialyzer and clearance calculated in the conventional way as the broken line against perfusion rate using the arterial concentration as the mid point between the arterial and the venous side

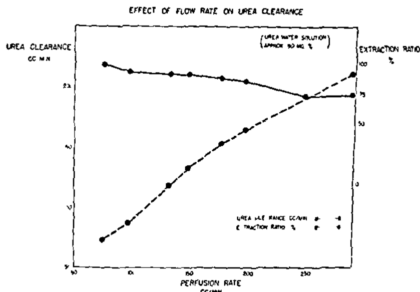


FIGURE 68 Reprinted by permission from Merrill J P *et al* The use of an artificial kidney I Technique *J Clin Investigation* 29 412 (1950)

since it changes of course in transit As might be expected when the perfusion rate of blood through the apparatus is increased the extraction fraction is decreased to some extent On the other hand the total metabolites, the total solute removed is very nearly a linear function of the rate of flow through the apparatus In terms of total removal over a given period of time this apparatus should certainly be operated at rapid rates at least with the area and with the type of membrane in use at present

Another interesting fact is that although one needs constantly to refresh the bath it is not necessary to have a large volume of bath fluid in contact with the membrane These are extremely rapid transfers for small molecules and all that is needed is a thin film of bath fluid on the outside of the membrane With the rotating type of apparatus the drum is made of wire screening and as the drum rotates bath fluid is picked up in the interstices so that although 60 per cent of the apparatus is above the surface of the bath at any given time that thin layer of film is enough to accomplish almost the same thing as when the drum is totally immersed This can be seen in Table VII which gives the results of an experiment in which an urea solution 89 5 milligrams per cent urea nitrogen (the osmolarities of the bath and urea solution were balanced) was run through the apparatus at a rate of 200 milliliters

per minute When the bath pan was just touching the bottom of the drum about 12 per cent of the drum circumference was immersed with the bath fully raised about 40 per cent was immersed and halfway about 25 per cent Despite the rather marked difference in circumference actually exposed to the water in the bath (but not of course to the water contained on the top of the drum)

TABLE VII
Extraction Ratios
at Varying Degrees of Drum Immersion

		% of drum circumference immersed	Urea extraction ratio
A	Bath Fully Raised	40%	90%
B	Bath Raised Half way	25%	88%
C	Bath Touching	12%	85%

Osmolarity of Urea Solution and Glucose Bath Solution = 33 mos/liter
Urea Solution = 89 5 mg % Urea Nitrogen
Flow = 200 ml/minute

there was very little change in extraction fraction perhaps within the limit of error of the experiment so this is a rapid process and it does not necessarily need to be carried on with tremendously large volumes of fluid so long as that bath fluid is constantly and adequately refreshed

This Conference I understand should bring out problems and certainly we have our share with artificial kidneys Interestingly enough one of them is the fact that the artificial kidney at least the Kolff type of apparatus – and we have used all three – is perhaps too efficient If one considers that in treating a patient with chronic renal insufficiency which has occurred gradually over a period of years and in which undoubtedly some physiologic compensatory mechanics have developed one attempts to change the blood chemistry – which is all we know we are doing – in a matter of six hours I think one might well expect to upset some physiologic compensatory mechanism

Table VIII is the record of a patient with kidney disease who had nitrogen retention for a period of ten years slowly progressing She came into the hospital comatose in convulsions In a six hour run with the artificial kidney these chemical changes (Table VIII) occurred Blood urea nitrogen dropped from 223 to 43 mgm per

TABLE VIII

B.B. ♀ Age 53 Run # 137 10/24/50 Dx Polycystic Kidneys

	0 Hour	*6 Hour*	*Normal*
B U N	223	43	10 - 15
Na	119	132	137 - 143
K	4 0	3 6	3 5 - 4 5
CO_2	7 2	17 1	22 - 31
Cl	92	107	99 - 111
P	4 1	1 4	0 8 - 1 45
Sugar	128	145	80 - 120
Hct	25	23	45

Bath Urea Nitrogen — 51 Gm Removed

cent serum, sodium rose from 119 to 132 mEq per L The serum K was not particularly high and changed very little The carbon dioxide content rose from 7 2 to 17 1 mEq per L And other changes of that order of magnitude occurred in this period of six hours Although this particular patient suffered no ill effects, I think it should give one pause in considering the different time relationships between the development and the correction of the chemical changes we are measuring

Figure 69 shows the changes observed in another patient with chronic renal insufficiency in whom the blood pH was followed and the CO_2 content, the pCO_2 and buffer base derived from the nomogram of Singer and Hastings(5) Although this Table does not show it quite as markedly as in some others, it can be seen that there was a rapid change in serum pH, with a drop of the pCO_2 below the normal range Although one cannot say, as Dr Hastings points out, that this situation is any worse than if these two points were exchanged, the rapidity of change should give one pause We have seen at least one situation in which the pH continued up to 8 0 and the pCO_2 continued to drop Such a situation may be thought of as analogous to that in the patient with diabetic acidosis who is treated with large, rapid infusions of sodium bicarbonate and in whom a transient respiratory alkalosis may apparently be produced(5) We believe this sort of change should occur over a period of, perhaps, twelve to fourteen or twenty-four hours rather than six hours

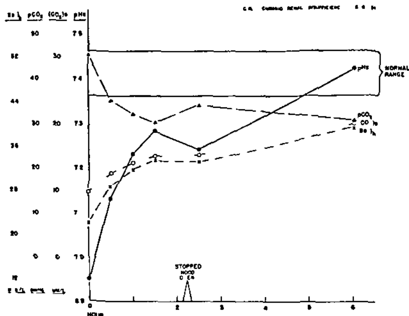


Figure 69

Mudge In a run of this type what is the buffer in the bath?

Merrill It is CO bicarbonate Phosphate of course might be and is a more effective buffer but since one of the difficulties these people have is high phosphate levels we leave it out of the bath in order to increase the diffusion gradient

Mudge And how much bicarbonate and CO are in the bath?

Merrill The bicarbonate concentration of the bath is 26 millimols per liter The atmosphere is 10 per cent CO 90 per cent oxygen

Chinard What is the pH of the bath fluid?

Merrill It is about 8 4 After exposure to CO it drops to about 7 4

Berliner It should be lower than that With 10 per cent CO the pH should be down around 7 1 or 7 2

Pappenheimer He is right apparently it does not saturate

Merrill No You see this is not a completely closed system and some of the CO does leak out For that reason there is incomplete saturation That is the reason why CO has to be constantly added

Pitts Is the bath fluid hypertonic? How do you prevent changes in blood volume?

Merrill The bath fluid we are using at the present time with 200 milligrams per cent of glucose contains about 305 milliosmols per liter That can be changed by changing the electrolyte content and by adding glucose but that is a separate problem which perhaps we can discuss later

In the totally anuric patient who as far as we can determine with careful observation has no negative external balance of sodium chloride the serum concentrations of sodium and chloride drop with prolongation of anuria Whether that is due to expansion of the extracellular space as has been intimated by some observers or whether it is due to intracellular movement of sodium and chloride or both I do not know but I am not sure that it is physiologic to take a patient with such a hyponatremia and bring the extracellular fluid levels up to normal rapidly in a period of six hours We have some evidence which is not yet complete that it may not be However that is a point which is still debatable

Too rapid correction of the chemical abnormalities may induce cardiac arrhythmia The patient with severe renal insufficiency is particularly susceptible to these disorders during digitalization because of the rapid changes in potassium and rapid increases in potassium antagonists such as calcium and sodium The relationship of digitalis and calcium in the causation of arrhythmia has been hinted at for some time In a patient who has some elevation of the serum potassium elevation in the serum sodium and correction of other chemical abnormalities may change these things in the opposite direction and lead to an actual tachycardia We have also seen ventricular tachycardia The same thing happens in the anuric when the serum potassium is reduced (Figure 70) In the human this has been controlled but in a dog whose serum potassium has been dropped rapidly with the artificial kidney the digitalizing dose is reduced to one tenth the usual

When we first became interested in the problem of the treatment of the patient with nitrogen retention with the artificial kidney it did not appear feasible to treat chronic uremia because it seemed reasonable to believe that nitrogen excretion depends on a high serum concentration of nitrogen Reduction in serum nitrogen by dialysis rapidly reduced the blood urea nitrogen but the urea nitrogen excretion diminished during the following days rather markedly and the two then increased in proportion Hence from that standpoint alone it did not seem to be a good procedure However an interesting clinical phenomenon became apparent if one treats a patient with chronic severe renal insufficiency (as the

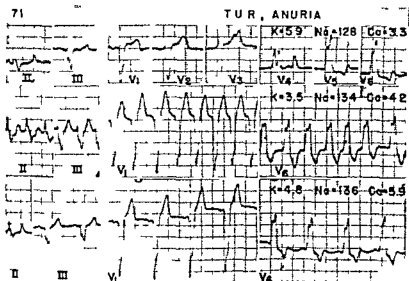


FIGURE 70

case shown in Figure 71 whose CO was 7 8 mM per L and the blood urea nitrogen 160 mgms per 100 ml prior to dialysis) clinical response to dialysis does not become apparent in terms of beneficial results for a period of from twenty four to thirty six hours despite chemical adjustment That is different from the situation of the patient with acute renal insufficiency whose clinical improvement may become manifest during dialysis or within eight to ten hours after the run And interestingly enough the clinical improvement in the chronic case becomes manifest as the chemical changes revert to the predialysis level and may persist in such patients for a month or more in spite of this reversion

Figure 71 is the record of a patient treated conservatively for a period of ten days before dialysis The serum CO with which she came in was 9 8 mM per L and the predialysis level was 7 8 The hematocrit was raised by transfusion of packed cells the blood urea nitrogen fell sharply with dialysis Here 51 6 grams of urea nitrogen were removed within a six hour period All values returned to the predialysis levels within about five days but in spite of that her clinical improvement persisted This patient was subjected to dialysis on three occasions On the third occasion urea was placed in the bath fluid so that the postdialysis serum levels of urea nitrogen were identical to the predialysis levels and as suspected it

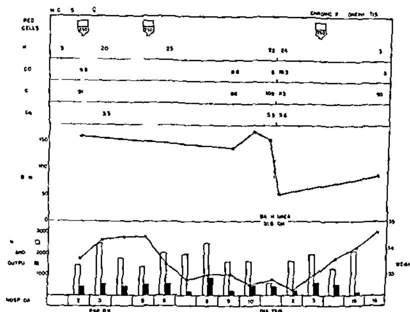


FIGURE 71

made no difference whatever in her clinical improvement

We feel that the artificial kidney does have a place in the management of these patients

Shannon Could you tell us the particular clinical improvements?

Merrill This patient suffered from nausea vomiting stupor and convulsions The most striking improvement was in the nausea and vomiting as well as in the level of awareness which went from almost complete stupor to sitting up and talking to relatives and eating

One of the interesting implications of the fact that the artificial kidney has no tubules is shown in Figure 72 the chart of a patient with bromide intoxication Bromide has a predominantly extra cellular position because of its chemical similarity to chloride It is handled by the renal tubules in somewhat the same fashion It is not a foreign substance in the sense that it is rapidly excreted Indeed in some of the older literature it was stated that perhaps the chloride was more readily excreted than the bromide I am not sure that has been borne out by recent work but in any case bromide is certainly filtered and reabsorbed and is a toxic substance in higher concentrations If one could eliminate tubular reabsorption it could be easily excreted This can be done with a cellophane membrane by maintaining the diffusion gradient

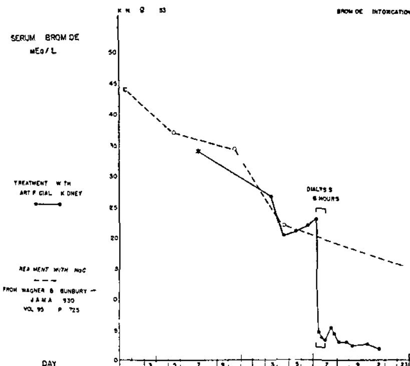


FIGURE 72

across the membrane so that no net reabsorption occurs Serum bromide concentrations observed by Wagner and Bunbury(6) in a patient with bromide intoxication (broken line) who was treated with saline solution which is the conventional treatment are compared in Figure 72 with those of a patient who was treated with the artificial kidney (solid line) For the first few days they are roughly similar but on the seventeenth day dialysis caused a remarkable decrease in bromide concentration It has been postulated that one should not attempt to effect excretion of bromide rapidly because in some mysterious way it harmed the patient It certainly did not harm this patient There was remarkable clinical improvement She had been totally disoriented for seventeen days prior to dialysis and the morning following it she was completely reoriented with amnesia for this whole period

I hope I can get some help from the Conference Group concerning the problem of controlling the osmotic pressure of the bath In many patients the serum osmolarity may be as high as 350

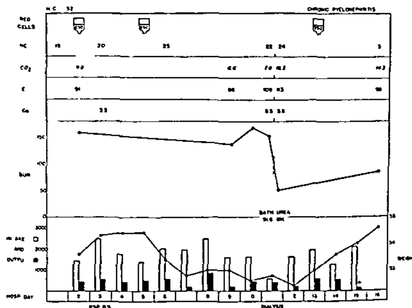


FIGURE 71

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Merrill This patient suffered from nausea vomiting stupor and convulsions The most striking improvement was in the nausea and vomiting as well as in the level of awareness which went from almost complete stupor to sitting up and talking to relatives and eating

One of the interesting implications of the fact that the artificial kidney has no tubules is shown in Figure 72 the chart of a patient with bromide intoxication Bromide has a predominantly extracellular position because of its chemical similarity to chloride It is handled by the renal tubules in somewhat the same fashion It is not a foreign substance in the sense that it is rapidly excreted Indeed in some of the older literature it was stated that perhaps the chloride was more readily excreted than the bromide I am not sure that has been borne out by recent work but in any case bromide is certainly filtered and reabsorbed and is a toxic substance in higher concentrations If one could eliminate tubular reabsorption it could be easily excreted This can be done with a cellophane membrane by maintaining the diffusion gradient

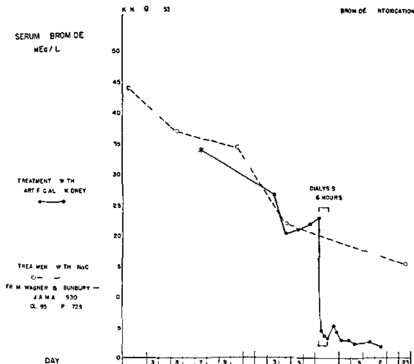


Figure 72

across the membrane so that no net reabsorption occurs Serum bromide concentrations observed by Wagner and Bunbury(6) in a patient with bromide intoxication (broken line) who was treated with saline solution which is the conventional treatment are compared in Figure 72 with those of a patient who was treated with the artificial kidney (solid line) For the first few days they are roughly similar but on the seventeenth day dialysis caused a remarkable decrease in bromide concentration It has been postulated that one should not attempt to effect excretion of bromide rapidly because in some mysterious way it harmed the patient It certainly did not harm this patient There was remarkable clinical improvement She had been totally disoriented for seventeen days prior to dialysis and the morning following it she was completely reoriented with amnesia for this whole period

I hope I can get some help from the Conference Group concerning the problem of controlling the osmotic pressure of the bath In many patients the serum osmolarity may be as high as 350

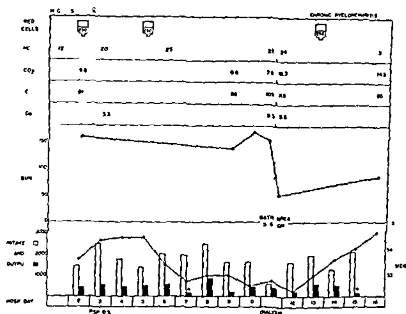


Figure 71

made no difference whatever in her clinical improvement

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we use, is nearly a gel It is an extremely uncomfortable and sticky liquid to handle

Darrow Have you ever tried dextran?

Merrill No, we have not

Shannon One would not expect any net change in osmolarity when such large molecular species are used For example, the dextrans utilized as plasma expanders have a mean molecular weight in the order of magnitude of 50,000

Merrill Of course, that is the point The osmotic efficacy varies inversely with the molecular weight For these substances, the statement has been made that they consistently maintain whatever osmotic pressure they are exerting because they do not diffuse in On the other hand, since the glucose does diffuse in, it has not been a practical way of doing it

Shannon The osmotic pressures of the concentrations of glucose utilized are small

Merrill Yes, extremely small But because of that and because it is extremely difficult to handle, it has not been a practical way of doing it

Chinard Have you considered using polyvinylpyrrolidone which is cheaper, I believe, than dextran?

Merrill It still has a large molecular weight

Berliner Is this a matter of balancing osmotic pressure or oncotic pressure?

Merrill Both, initially

Berliner The osmotic pressure of the blood as estimated from the freezing point will include a large amount of urea in these patients For purposes of physiological regulation I think the urea should probably be disregarded Actually, if the total osmotic pressure of the bath fluid is the same as that of normal plasma and if, at the same time, transfer of fluid is prevented due to the oncotic pressure of plasma proteins I think you would end up where you would like to be

Leutscher I wonder whether you could get around the problem of making a high concentration of small molecular weight material available in the bath without having too much transfer into the blood by blocking off the path of the last turn of cellophane tubing and putting a high glucose concentration in this path so that only the last loop was exposed to it? In that way, I think you could probably move water out much faster than the glucose would diffuse in as Dr Pappenheimer indicated previously The shorter time

milliosmols per liter, and their blood is dialyzed against a stock bath which contains only 305 milliosmols per liter In the early days, Dr Kolff and others working with the artificial kidney attempted to control this discrepancy by adding glucose to the bath in concentrations of 2000 to 3000 milligrams per cent, thereby increasing the osmolarity of the bath, attempting either to dehydrate the patient by exchange of water or to prevent hydration as a result of the difference in osmolarity At first we measured the patient's serum freezing point and then constructed our bath so that the osmolarity of the bath, made up with glucose, balanced or overbalanced that of the patient and no net water exchange could occur But I think it has become apparent from what Dr Pappenheimer has said that this is not a reliable solution As the blood passes along the cellophane membrane, the osmolarity changes It doesn't change much in the bath since the volume is 100 liters, but glucose does diffuse to the blood Hence, the system is changing unpredictably

Fremont-Smith What happens to the freezing point of the bath at the end?

Merrill The freezing point of the bath does not change much because the bath is large and is refreshed every two hours with additional glucose The plasma glucose concentration changes remarkably Patients in whom we used 2000 and 3000 grams of glucose did not do well They became drowsy and their blood sugar levels rose to 700 or 800 mgm per cent In any case, the serum osmolarity tends to drop over a period of six hours, apparently because there is net transfer of water into the patient at the beginning of dialysis that decreases toward the end It has not been a dangerous way of treating the patient in terms of water transfer Dr Alwall(7) also found this an ineffective way of removing water in some experiments on rabbits using the same concentration of glucose in the bath

One of the things that immediately suggests itself is to use a larger molecule which stays in the bath and maintains its osmotic efficacy We have tried acacia, gelatine, globin, and bovine albumin but the difficulty with all of these things is that they are rather expensive and, at least in the case of gelatine, the molecular weight varies greatly in addition to its being a substance that, weight for weight, is less effective osmotically, than glucose The main difficulty, of course, is the viscosity A 5 per cent solution of gelatine in 100 liters of bath water at a 100° temperature, which is what

we use, is nearly a gel It is an extremely uncomfortable and sticky liquid to handle

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of exposure would prevent equilibration of glucose with loss of osmotic effectiveness

Merrill That is an interesting suggestion It would involve a little change in design

Luetscher In the presence of a large excess of water, it might well be advantageous to remove some

Merrill That is our practice in the anuric patient Attempts have been made to remove water by using filtration, i e, by increasing the hydrostatic gradient across the membrane This has been done with the Alwall apparatus(8) and with the Skeggs and Leonards apparatus(9) Our experience has been limited simply to plasma-water content in one passage It is possible, by increasing the filtration pressure or the hydrostatic pressure within the membrane, to move water and to concentrate nontransferable solute, particularly protein Is this of practical clinical effect? Can one utilize it to treat patients with intractable edema? Unfortunately, we know so little about the causes and cure of intractable edema that I am not sure it is wise to approach it by as violent a method as this But it has been tried Alwall has tried it in cardiacs with uremia and edema, and his conclusion is that filtering at a pressure of about 180 millimeters of mercury over a period of eight to twelve hours is not a practical way of doing it The experiments of Dr Leonards in which he used his apparatus as a pure filter are interesting He applied negative pressure on either side of the cellophane membrane, and, in dogs, he found it was possible during the period of his experiments to remove 2000 to 2500 ml of filtrate in that way If suitable salt solutions were used to replace electrolytes the dogs were not harmed But I think the most significant of his studies were made in animals in whom edema was created by hypoproteinemia by *low protein diet and plasmapheresis*, and in animals with pericarditis ascites and edema induced by the introduction of a Dakin's solution in the pericardium His conclusions were that no more fluid could be removed from these dogs than from normal dogs before death Indeed in a number of his dogs, death was caused in an attempt to remove fluid by this method, and the amount of edema fluid which could safely be removed was quite limited

We had two experiences very early in the course of our work with the artificial kidney in which we attempted to remove edema by using low sodium and high glucose concentrations in the bath, one a cirrhotic patient and one a patient with edema of essentially unknown origin These patients certainly lost weight and they lost

water, but they did it by mechanisms which we had not expected They had no renal insufficiency One who had edema on the basis of cirrhosis, lost, in a period of two and a half hours, a total of about 2900 ml of fluid, but all of it by way of his own kidneys A tremendous diuresis was produced Our first inclination was to think that this was simply an osmotic diuresis due to glucose loading The blood sugar rose during the course of that experiment from 130 to 169 mgms per cent he developed acute salt depletion cramps, and the experiment was terminated Unfortunately, we were not able to decide whether the diuresis was simply a function of the osmotic diuresis The urine flow did rise from a control of 2 ml per minute to 27 ml per minute at the height of the diuresis, but, interestingly enough, his urine sodium concentration also rose in direct proportion, from about 4 milliequivalents per liter to 80 milliequivalents per liter at the peak of the diuresis We did not have measurements of filtration rates, but it is interesting to compare these observations with the data of Wesson and Anslow(10) in studies of mannitol diuresis in which the urine flow increased to approximately the same levels we obtained in this gentleman In their experiment, however the urine sodium concentration decreased successively with increased urine flows We did one other thing with this patient We had been interested in cardiac outputs and blood pressures, and we measured the cardiac output with a catheter in the pulmonary artery, employing the Fick principle During the period of diuresis, it almost doubled, so that while I can say nothing about his renal hemodynamic changes, I think it is interesting to speculate on two facts first the cardiac output was double during that period, and, second, that with increasing urine flows, the urinary sodium concentration increased almost in direct proportion to that increase in flow

Barnett Were the plasma proteins reduced?

Merrill Yes, they were The sodium concentration, however, did not rise from normal

We have had one other patient in whom we did measure PAH and glomerular filtration rate during a similar situation I don't know that we can say that the filtration rate showed a progressive increase because it was calculated in the face of falling plasma levels, I think probably we will have to throw them out as a measure of actual filtration rate It is an interesting observation, and I have no idea what it means But we did not dehydrate these patients by means of transfer of water across the membrane, and I would be happy to hear any interpretation of those data

Selkurt Is it possible that ADH and adrenal cortical hormones

are removed during dialysis and that this might influence the renal mechanisms? As I understand it, no attempt was made to add to the perfusion fluid the quantities of these hormones which might have been lost

Merrill No we certainly did not Can you tell me what the molecular weight or structure of these hormones are?

Shannon The antidiuretic hormone is composed of some 11 amino acid residues Presumably, it would not diffuse across your membrane with any rapidity

Merrill Yes that would be my impression

Mudge Will steroids go across?

Merrill I shouldn t think so As I have said, we have been unable to demonstrate the fact that inulin goes across, although I am sure it does *in vitro*

Shannon It is hardly likely that the steroids will

Swann They are pretty well bound to proteins They don't go out

Thorn There is no evidence of acute hormonal deficiency in any of the uremic patients immediately following dialysis We have been very concerned with that particular problem

Berliner The data in Leonards' experiments seem to suggest that dogs cannot get along without the excess fluid Dr Davis* in our laboratory has been working with a similar preparation dogs with constrictions of the thoracic inferior vena cava These animals develop a large volume of ascites Upon adrenalectomy and removal of all substitution therapy they will diurese over a period of two weeks and remain in perfectly good condition until all the ascitic fluid has been exhausted That suggests that, at least in this preparation there is no need for the extra fluid if there is some way of getting rid of it

Chinard I should like to ask about the effect of dialysis on amino acids

Merrill In an effort to determine whether or not we were removing nutritionally significant amounts of things like amino acids, we have, with the help of Dr Christensen, done amino acid nitrogen assays The normal plasma level of amino acid nitrogen is about 4 milligrams per cent, and it is changed very little by dialysis, but the amount of alpha amino acid nitrogen recovered in the bath, on the basis of extracellular volume calculations, is very large In one such situation the change in blood level was only 12 per cent of what was expected from the amount of amino acid nitrogen found in the bath and extracellular fluid volume (Figure 73) On

* Davis J O and Howell, D S Personal communication

the other hand in liver disease when there is a high plasma concentration of amino acid nitrogen the plasma level drops down to normal and then in spite of continued removal in the bath remains about normal (Figure 73) That looks as if a homeostatic mechanism were operating to maintain the extracellular concentration

REMOVAL OF ALPHA AMINO ACID NITROGEN

PATIENT		NORMAL LIVER FN	HEPATIC INSUFFICIENCY DC	HEPATIC INSUFFICIENCY DC	HEPATIC INSUFFICIENCY MR
SERUM LEVEL MG %	INITIAL	3.99	6.35	5.9	60
	FINAL	3.6	4.3	4	3.74
	DECREASE	38	2.2	102	86
TOTAL REMOVED MGS	CALCULATED FROM EC FLUID	76	403	94	364
	FOUND IN DIALYSATE	590	420	2.0	633
FRACTION FROM EC FLUID		2 %	96 %	92 %	4 %

Figure 73

This thing is also true for potassium as Reinecke(11) has demonstrated in dogs and as we have shown in both dogs and humans In dogs we cannot with good flows and with large amounts removed in the bath during eight hour runs reduce the serum potassium level below 2 milliequivalents per liter In the human if one takes a patient with a high initial level perhaps 8 one finds that the value may drop to 3 or 4 mEq per L and then in spite of the fact that 300 or 400 per cent of the extracellular potassium content may be removed it remains right there The people in Minnesota working with dogs investigated levels in the venous blood coming from the hindlimb and they found a higher concentration They attributed it as I think we should perhaps to a replenishment of the extracellular level by some homeostatic mechanism causing transfer of potassium out of the cells Dr Christiansen feels that the amounts of amino acids removed are not nutritionally significant and he assures us that we do not have to worry about it

Clinard Although it looks nice on a chart to have an abrupt drop in blood urea nitrogen is there evidence that urea itself

causes any of the symptoms seen in uremia? With respect to the amino acid nitrogen levels as you have pointed out, they are not generally elevated even in the terminal stages of chronic glomerular nephritis There is evidence that some other nitrogenous products are present, occasionally in very large amounts, in the blood of patients with uremia Kirk, using a nitrous acid method, found levels of amino" nitrogen as high as 30 mg per cent in some individuals With the gasometric ninhydrin method that Dr Christiansen uses, the α-amino nitrogen is much lower than that Possibly these products are removed by the dialysis It would be of interest to determine the nitrous acid reacting material in the blood of your patients before and after dialysis, the removal of these substances might have some bearing on the clinical improvement obtained

Thorn I have felt for some time that if we had a highly purified urea solution available at relatively low cost, with the ammonia contaminant eliminated, it might be desirable to include urea in the bath and thereby maintain a constant urea level in the blood Under these circumstances, the net change in the nonessential components of the system might be much greater, and the effect of massive reduction in blood urea would not complicate the situation We have not been able to carry out these experiments with any frequency because of the necessity of employing highly purified urea

Shock In our laboratory Dr Miller has been using intravenous infusions of recrystallized urea to raise the blood levels to 150 to 180 milligrams per cent without any clinical symptoms appearing in these people at all

Merrill It is certainly our impression, and that of others that the older literature attributing toxic effects to urea was probably based on the dehydration that resulted, or perhaps the ammonia contamination rather than on the urea itself

Shannon There are a lot of contaminants in the ordinary urea

Merrill I think that may be the answer

Shannon Some of them are very toxic But with suitable recrystallization, particularly with complete removal from contact with such things as metal suitable preparations can be obtained

Merrill In many patients we have seen oliguria following dialysis It does not appear in all, but in patients who have some degree of decrease in their urinary volume initially and, perhaps, a preponderance of glomerular rather than tubular damage The urine concentration may be a little higher than usual and the urinary pH very low Most have some degree of hypertension A typical

situation was one in which a patient who was put on a constant fluid and sodium intake for a period of three days had an urine volume of about 900 ml The patient was then dialyzed the urinary volume dropped off rather sharply, to about 400 or 300 for a period of four days, and then rose again to the control levels the intake being maintained constantly during this period The total osmolarity of this patient's serum was something like 360 mosM per L Following dialysis, the urea dropped off sharply and the plasma sodium and chloride rose The total serum osmolarity fell by about 70 mosM per L, and with that the urine sodium and chloride which had been in the range of 8 to 10 mEq per L, increased and the urea previously 150 mosM per L, decreased With the resumption of urine output, all these things tended to come back to the predialysis level By raising the plasma levels of sodium and chloride and by reducing the blood urea nitrogen we are imposing a hypertonic load of reabsorbable ion and decreasing the load of nonreabsorbable substance, urea, which might be contributing to the urine volume

The other point that interests me is whether by increasing urea which should have very little effect on the production of antidiuretic hormone and by rapidly increasing the plasma sodium chloride we induce an antidiuretic response which may have some effect when some degree of tubular function remains with very little filtration

In a certain number of our patients the blood pressure tends to rise during the course of dialysis This response does not occur until an hour or an hour and a half after the start of dialysis It is associated with a rise in both systolic and diastolic pressure and with some increase in pulse rate Previously hypertensive patients have an increase in peripheral resistance without much change in the cardiac output This change is independent of the plasma volume, at least as measured on two occasions It is independent of weight and, apparently as far as we could judge, of rate of hydration This response also occurs in hypotensive patients even those who have been refractory to volume replacement with blood plasma, and the like (Figure 74) This response appears to be to some extent, a function of flow through the apparatus, and, since flow appears to be a function of clearance we first thought that we might be removing something which was responsible for vasomotor activity We have talked very little about the idea recently and I don't intend to go into it here

Is this simply a mechanical effect? There is a kind of arteriovenous fistula here and certainly an arteriovenous fistula will raise

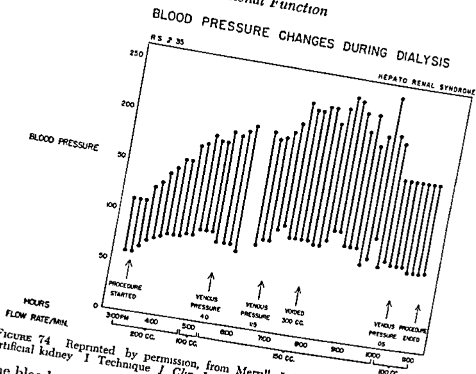


Figure 74 Reprinted by permission, from Merrill J P, *et al* The use of an artificial kidney I Technique *J Clin Investigation* 29, 412 (1950)

the blood pressure However, the apparatus does not make a direct connection between the artery and the vein Also, the blood pressure does not rise immediately, and, finally, it does not drop until four to six hours after the apparatus has been disconnected The diastolic pressure increases rather than decreases, which is not typical of an arteriovenous fistula Therefore, we do not feel this is the answer, but, rather, that it is in some way related to the procedure of dialysis

The question has been vigorously raised by at least one individual as to whether there is something in the cellophane which is causing this response, and it is an interesting problem There are people working with dogs who point out that if you do not wash or boil the cellophane adequately, over a period of time, you will certainly get a pressor response But there are a number of factors which would make me believe that this is not the complete explanation The first is that the pressor response you get in the dog with improperly prepared cellophane differs from the human reaction It occurs immediately and rapidly and then drops off The second thing is that improper preparation of the cellophane is almost as likely to give a depressor response, independent of volume,

as it is a pressor response Even with proper and careful preparation of the cellophane we get pressor responses The other thing that intrigues me is that even if it were something in the cellophane I wonder if it would not be worth investigating simply from the standpoint of being able to do this in a patient with refractory hypotension

Preston Does this blood pressure rise occur when blood is introduced into the artificial kidney from a vein and returned from the artificial kidney back into the venous system?

Merrill Because we have never used a venous system I dont know

Selkurt Will you state again what happens to the cardiac output during this phase?

Merrill Yes The hypertensive individual with severe congestive heart failure as might be expected is incapable of increasing cardiac output rapidly The blood pressure goes up as a function of peripheral resistance increase In the cirrhotic patient (Figure 75) and in other patients with refractory hypotension the blood pressure rise is a function of increase in cardiac output and I would say in most of the patients who were not in severe congestive heart failure the cardiac output does increase

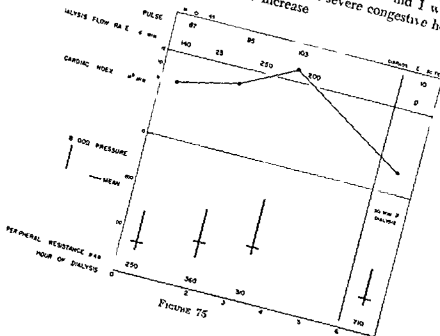


Figure 75

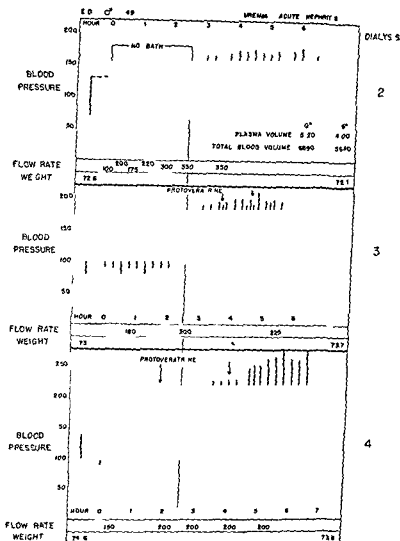


FIGURE 76

Figure 76 is the chart of a patient who was dialyzed on three occasions, and on those three occasions the blood pressure began to rise after about an hour to an hour and a half in the usual manner The drop which appears on the chart was due to protoveratrine administered during a study of the cardiac output On the third occasion the patient was run without immersing the cellophane prepared in the same way as on the other occasions in the bath There is a distinctly different type of curve there We have

done this in three patients with similar results It seems to me that if it were the cellophane or if it were simply a mechanical defect of procedure this third procedure should have given curves that look like the first two I haven t any idea why this slow rise occurs and I am not sure of what practical importance it is but I think it is probably worth investigating I am sure it does occur in a certain percentage of our patients and I am equally sure on the basis of what I previously said that it is not simply a toxic effect of the cellophane since we have been able to reproduce it after prolonged washing and boiling of the cellophane and since it does not occur unless the drum is immersed in the bath and dialysis is actually occurring

That is about all I have to say I would be most anxious to hear any possible explanations for the oliguria or to have suggestions as to whether we might help these people perhaps by giving them nonabsorbable substances like mannitol to maintain the urine volume after dialysis

Pitts What difference does it make what the urine volume is? Didn t you state that they would not excrete any appreciable amount of urea at reduced plasma urea levels? Is it not possible that this oliguria is just a consequence of low urea excretion?

Merrill That certainly would be my explanation for it and aside from the fact that it is a frightening thing to watch for the first four or five days I cannot honestly say that it has done any harm ince the urine flow apparently does not return until the blood urea itrogen levels have built up again

Selkurt In this last experiment did you do blood volume deter minations to see whether there is a positive fluid retention under ese circumstances?

Merrill Yes

elkurt Does it happen?

lerrill No it has not happened in the four we have measured

Pitts Is there any apparatus in which you could create a filtration pressure sufficient to overcome colloidal osmotic pressure?

Merrill Oh yes you can do that in any one of the filters either by dropping the tube in the bath at the desired length as Dr Alwall(2) does or by constricting the outflow You can raise the pressure to any degree you want inside the membrane

Pitts What is the efficacy of the Kolff apparatus in relation to the others?

Merrill The Kolff apparatus is about twice as effective as the Westinghouse type of Alwall apparatus Again I would emphasize that I am not sure that this is really desirable Perhaps if we could keep

some of these patients on from twelve to twenty-four hours, rather than six hours, the homeostatic mechanism might be better served

Winton If you restrict the outflow of the Kolff apparatus, doesn't the sausage skin blow up?

Merrill It certainly does, and for that reason you have to be extremely careful that the terminal loop is not dilated, otherwise, the patient can lose as much as 500 to 2000 ml of blood into the distensible cellophane tubing However, since the cellophane on the apparatus is kept tight by the rotation of the drum, it has a constant volume The remainder of the system is rigid, and as long as the capacity of the outflow pump is not exceeded, this is not a serious problem The arterial inflow is not run at more than 300 ml a minute and the outflow pump will handle up to 700 ml per minute With increments of flow, the volume in the machine will increase by only 160 ml for each 100 ml increment and since we never deliver more than 300 ml per minute, this is not dangerous

Peabody What happens to the serum potassium of patients who have low serum sodium when you elevate the serum sodium?

Merrill It depends on the bath potassium concentration and, to some extent I suppose, upon how much glucose you are infusing into them from the bath

Darrow How do you know which way you want the potassium to go?

Thorn I believe Dr Darrow is implying that the total body content of potassium in a patient may not be reflected by the serum level Thus even in the presence of a marked elevation in serum potassium, there may actually be a total body depletion Under these circumstances, you would probably not wish to drop the serum value much below 6 with the dialysis

Merrill I think that is an extremely important point The syndrome of potassium intoxication may be independent of the given level of potassium With Dr Moore's help, we have studied the K^{42} space in two patients Both of these patients had severe potassium intoxication in terms of clinical syndrome, but one of them had 35 per cent, and the other 40 per cent depletion of total body potassium as measured by the isotope dilution

Pitts How do they respond to glucose alone?

Merrill If given glucose and insulin, the potassium concentration will drop The level decreases for about four to six hours and then back up again it comes In the anuric patient, in whom this is a problem, you must give more glucose and insulin, which involves more fluid and you soon find yourself at a point of diminishing

returns If under the same circumstances, you dialyze such a patient, the level comes down sharply and it may stay down for four or five days and then rise

Darrow What do you do about calcium?

Merrill We have about 2 milliequivalents of calcium in the bath assuming that is, roughly, the level of ionized or diffusible calcium that we like to prevent from moving out

Darrow What do you do about phosphorus?

Merrill Since these people have a high phosphate level we like to attempt to remove phosphate so we put none in the bath

Grafflin Have you ever run a dialysis without any added calcium in the bath?

Merrill No we haven't

Pitts When and how do you use antibiotics in this system?

Merrill We give these people penicillin if they are not already on it, for a day before and a day afterward Occasionally one of them may have a little stitch infection but it has never been a serious problem

Darrow What do you do about food? Are they able to eat perfectly well during this process?

Merrill Oh, yes, but most are too ill to eat They are given glucose, as much glucose as we can get into them, within the volume of fluid to which we are restricted

Darrow That doesn't constitute adequate nutrition

Merrill No, it certainly does not and that brings up an entirely different point, that is, the treatment of the anuric patient As far as the caloric requirement goes, first of all you must have at least a hundred grams of glucose, and the more glucose you can get in, the better However, you are restricted by the volume of fluid in which you can put that, and you cannot give it in amounts adequate to satisfy caloric need, unless you do as Dr Kolff did which was to give it into the right ventricle You cannot give the patients 50 per cent glucose daily Therefore, we get in at least a hundred grams and as much above that as we can, paying proper regard to the volume of fluid to which they are restricted and the total caloric requirement But if the anuria is reversible, it is a relatively short term situation and these people can draw on endogenous fat stores I don't have any evidence that they will burn exogenous fat in the form of custard powder, butter, and sugar mixtures given by mouth, rather than their own stores This does not apply to the patient with chronic kidney disease

Thorn In most patients with renal shutdown, I do not believe that

maintaining an adequate nutritional balance is a highly important issue, certainly not for the first few days of renal shutdown The most important problem is whether the underlying cause of renal shutdown is associated with continuing tissue destruction or the presence of massive tissue destruction Under these circumstances, the rise in blood urea nitrogen, potassium, and other components will be very great, and it is impossible to stem this tide with the addition of carbohydrate or fat The patient who has a simple renal shutdown may be demonstrated to have a relatively slow rise in blood urea nitrogen, and the rate of this rise can be definitely influenced by maintaining a high level of caloric intake of non-protein foods However, the really serious cases, those with massive tissue destruction, infection, or continuing breakdown of tissue protein due to any reason whatsoever may not be carried by conservative medical means since, if the kidney fails to open within a reasonable period of time, they may die from potassium intoxication or other metabolic disturbances There appears to be a tremendous latitude in the quantity of retained substances, at least as reflected by the blood urea nitrogen, which may be tolerated by patients under certain circumstances We have observed patients in terminal uremia with a blood urea nitrogen of 400 mg per 100 ml who were still alert, talking, and interested in their surroundings

My conclusion, then, is that unusual dietary supplement is only required in those patients with very prolonged periods of anuria, that is, over seven to ten days, and that in the more acute fulminating cases the correction of infection or of the cause of continuing metabolic breakdown of tissue is a much more important factor in the survival of the individual than is the supply of additional calories of carbohydrate and fat

Swann Going back to the hypertension that occurs, could it be interpreted in this way? The patient is slowly bled from an artery into the low pressure bath This reduces the peripheral resistance But at the same time, you are transfusing him with his lost blood In order to compensate for the reduced peripheral resistance, the patient calls into play the various pressor mechanisms available to him His cardiovascular system reacts as if it were in trouble But actually he is not in trouble because you are retransfusing him Because the transfusion is added to a reaction against low peripheral resistance, the blood pressure slowly rises A critical test of this theory would be the absence of hypertension in vein to-vein transfusions

Merrill Isn't that another way, Dr Swann, of simply stating that he has an arteriovenous fistula?

Swann: No, I think not, because you are not carrying over any of the arterial pressure into the venous side of the circuit.

Selkurt: In that connection, how does the heart rate change? Does it increase as the pressure comes up?

Merrill: It increases to some extent, but it is a variable increase - a constant increase in blood pressure.

Bradley: Did you ever encounter this effect during intestinal lavage or peritoneal lavage?

Merrill: No, but again I think that this procedure is so much more efficient than intestinal lavage that it may be a question of rate of exchange. In two patients we have seen pulmonary edema following dialysis without any change in blood volume. This has also been reported in two other instances by others (12, 13).

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TUBULAR SECRETION OF POTASSIUM AND ACID*

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TWO YEARS AGO when we discussed the tubular secretion of potassium and acid(1), it was on the premise that these were two separate and independent mechanisms which in certain fundamental aspects were similar Actually, at that time there was a large amount of clinical and experimental data, the latter largely the work of Dr Darrow(2), which should have suggested that the two mechanisms were indeed interrelated Furthermore, had we been alert to seek such an interdependence a clue to its nature was to be found in some of the data presented, namely, the rise in urine pH and bicarbonate excretion which accompanied the excretion of potassium salts More recently, among the effects of a compound designed specifically for the inhibition of the renal mechanism for secreting acid there appeared such a striking one on potassium excretion as to make the relationship between potassium metabolism and acidification apparent(3) In addition, the effects of this inhibitor on acidification itself have included certain outstanding findings, in large measure confirming the essential features of the mechanism worked out by Dr Pitts and his associates(4 5), but requiring revision of our views concerning certain important details

Dr Pitts has proposed(4) that the urine is acidified by a process in the distal tubule which consists of an exchange of hydrogen ions from the tubule cell for sodium ions from the fluid in the lumen The hydrogen ions for this process are made available through the metabolic activity of the cell by a mechanism in which carbonic anhydrase plays a central role There is reason to believe that all of the so called base-conserving mechanisms of the kidney are primarily dependent on this process since, by it, (a) bicarbonate is converted to carbonic acid and hence to CO_2 and water, (b) salts of weak acids are converted partially to their unionized acids, and (c) an acid medium is produced favoring the diffusion of ammonia thereto, this in turn permitting further secretion of hydrogen ion with a limited change in pH

* The work described in this presentation represents a joint effort of Drs Thomas J Kennedy Jr, Jack Orloff and the author

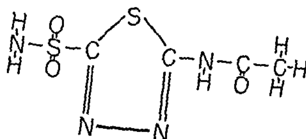
The process by which potassium is excreted is one with whose individual parts we have some familiarity but which has presented difficulty when it came to assembling these parts in their proper proportions We know that potassium is filtered at the glomeruli in amounts far larger than normally appear in the urine and that most of it is reabsorbed, presumably in the proximal tubule We also know that, at least under certain circumstances, potassium may be secreted, presumably by the cells of the distal tubule, and that this secretory process is one whereby potassium from the cell is exchanged for sodium from the fluid in the lumen, a situation analogous to that involved in acidification(6) What role this latter process may play in normal potassium excretion has remained obscure because of the confusion resulting from two tubular processes operating in opposite directions Some of the data to be presented may have a bearing on that question

We may consider briefly the evidence for the existence of a relationship between potassium metabolism and urine acidification It has long been known that in Cushing's syndrome depletion of potassium is accompanied by alkalosis(7,8) A similar depletion of potassium with development of alkalosis has been produced in patients(9) and experimental animals(2) by the administration of adrenal cortical steroids, and in rats by a diet simply deficient in potassium(2) Conversely, alkalosis leads to depletion of body potassium(2) On the other hand a high intake of neutral potassium salts tends to produce acidosis(6 10) while acidosis has been found to increase muscle potassium concentration(2), presumably by producing relative potassium retention (The latter is a point which has caused some question and discussion because of the obvious exceptions, I hope we shall have time to discuss it further later) *A priori*, in the absence of significant extrarenal losses, these [illegible]

tion has been available in (*a*) the rise in urinary pH which accompanies the administration of potassium salts (6,10), and (*b*) the excretion of acid urines in spite of severe alkalosis, which has been described in cases of gastric alkalosis and in ulcerative colitis with potassium depletion(11,12)

With this background, let us consider some of the effects observed when renal carbonic anhydrase is inhibited Because of the demonstrated involvement of this enzyme in urine acidification and hence in this phase of sodium reabsorption, a series of inhibitors has been

developed for possible use in cardiac failure(13,14) One of these, developed by American Cyanamid and having an *in vitro* activity some 330 times that of sulfanilamide, has been used in most of



2-Acetylamino-1,3,4 thiadiazole-5-sulfonamide

FIGURE 77 Reprinted by permission, from Berliner R W Kennedy T J Jr and Orloff J Relationship between acidification of the urine and potassium metabolism *Am J Med* 11, 274 (1951)

these studies The structure is shown in Figure 77 The effects o this compound are striking and apparently specific Although n the dog the urine becomes alkaline with doses as small as 5 mg per kilo, we have not observed that do

t ... produced almost instantaneously ... is administered, to obtain the full effect being that needed for the dead space of ureters and pelvis to be emptied

The typical effect is shown in Table IX This was a mildly acidotic dog receiving an infusion of phosphate so as to increase the excretion of titratable acid Immediately after the injection of 10 mg per kilo of #6063, the pH of t ... ne rose sharply, all titratable acidity disappeared and ... of bicarbonate was e ... traordinarily large amount return At the mome ... a ... t to which I shall increase in ... ium ... attention on the of potassiu ... o ... in the excretion every one ... c anhydrase in of conditio ... der a v variety of ... usion the only ex ... um

TABLF IX
Fffect of #6063 on Urine Acidification
Dog H Wt 26 kg

Time min	Urine Flow ml /min	Urine pH	Excr Titr Acid µEq /min	HCO_3^- Excr µEq /min	Excr Na µEq /min	Excr K+ µEq /min
77	Start infusion of Creatinine 25 mg /min Sodium Phosphate (pH 5 8) 200µM /min Sodium Chloride 1 mM /min Water 8 ml /min					
57	Priming Infusion Creatinine 2 0 gm Hydrochloric Acid 40 mEq					
0 21	0 81	5 58	105	0 2	85	68
21 43	0 77	5 41	154	0 2	85	61
43-64	0 94	5 38	164	0 3	116	52
73 79	Infusion #6063 (10 mg /kg)					
87 102	8 06	7 82	0	920 0	1654	264
102 117	6 21	7 80	0	750 0	1315	248
117 133	4 17	7 73	0	456 0	935	184

potassium excretion having been has been somewhat less striking initially high

The interpretation of this increase in potassium excretion depends on whether it is attributable to an increase in potassium secretion or to inhibition of potassium reabsorption In some instances the increment in potassium excretion was greater than the total amount being filtered requiring at least some increase in secretion However the margins were not impressive and the observation not sufficiently reproducible to allow any definite conclusion as to how the increase in excretion was effected In order to get a more definite answer we tried studying the effects of mercurial diuretics on the potassium excretion induced by #6063 An experiment of this sort is shown in Table X Following the injection of the mercurial the excretion of chloride and sodium go up as usual there is not much change in potassium excretion as is to be expected when starting from the level observed in the control periods here The effect of inhibiting carbonic anhydrase is added a marked increase in bicarbonate excretion covered by an equivalent amount of sodium occurs but essentially no change in potassium excretion Then the mercurial effect is removed by an injection of BAL The excretion of chloride returns to a low level with a parallel fall

TABLE X

Effect of salyrgan, No. 6063, and BAL on Electrolyte Excretion — Dog X — Weight 17 kg.

Time (min)	Urine Flow (ml/min)	Urine pH	Creatinine Clearance (ml/min)	Plasma K+ (mEq/L)	Plasma Na+ (mEq/L)	Plasma HCO₃ (mEq/L)	Excreted Na+ (µEq/min)	Excreted K+ (µEq/min)	Excreted Cl− (µEq/min)	Excreted HCO₃ (µEq/min)	Excreted Phosphate (µM/min)
69	Sustaining infusion Creatinine 11 0 mg/min, Na_2HPO_4 NaH_2PO_4 (pH 7 4)										
	169µM/min, NaCl 393µM/min, H_2O 15 ml/min										
[illegible]2	Priming infusion Creatinine 2 0 gm										
0 19	1 89	0 80	72	3 5	147	20	252	71	149	21	126
19 38	2 00	0 82	70	3 2	145	20	310	62	137	21	152
41	2 ml salyrgan theophylline i v, 1 ml salyrgan theophylline added to infusion										
64 74	8 51	0 90	70	3 2	116	18	1250	59	1010	70	162
	5	0 77	68	3 3	117	18	1390	60	1100	57	107
	3	0 83	67	3 0	115	18	1110	61	1250	12	105
	10mg/kg No 6063 i v 30 mg/kg/hr of No 6063 added to infusion										
	11 08	7 38	50	3 1	115	18	1700	68	1150	350	150
	10 41	7 39	57	3 1	116	10	1750	68	1150	381	105
	30	7 41	50		118	10	1070	60	1100	302	111
	mg BAL i m										
	75	7 82	58	3 0	116	18	300	171	72	351	123
	3 21	7 81	60	2 8			552	128	120	302	111
	3 00	7 63	70	2 0	118	17	641	130	147	371	111

in sodium excretion, and potassium excretion goes up to a level such as is usually observed after #6063 In other words, the increase in potassium excretion produced when carbonic anhydrase is inhibited can be prevented by mercurials

The question as to the effect of mercurial diuretics on potassium excretion has not been entirely settled However a consideration of the possibilities in this particular set of circumstances leads to the conclusion that this combination of effects increased excretion of potassium due to #6063 and inhibition of this increase by salyrgan could be produced only if the inhibition of carbonic anydrase led to increased secretion of potassium while this secretory process was in turn inhibited by mercurials

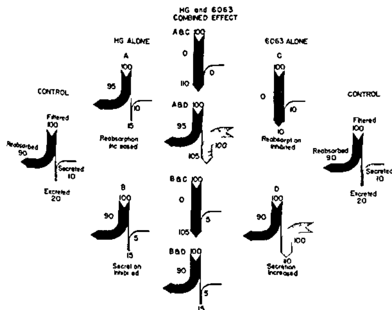


FIGURE 78 Reprinted by permission from Berliner R W Kennedy T J Jr and Orloff J Relationship between acidification of the urine and potassium metabolism *Am J Med* 11, 274 (1951)

Figure 78 may help to explain this conclusion This rather complicated looking affair is meant to show what the possible combinations of effects might be and is based on a single reasonable assumption, namely, that a mechanism knocked out by one drug will not be restored by one which ordinarily stimulates it The same

phone acid at the same rate The results have not been quantitatively impressive but in every instance except one potassium excretion fell as acidosis developed In the one exception potassium excretion did not change We are not prepared to take a definite stand on the question but it is our feeling that the effect of acidosis itself probably is to diminish potassium excretion

Mudge What are the changes in potassium excretion in absolute amounts?

Berliner From about 60 mEq per min down to 35 or 40 We are not starting from a very high level and it by no means goes down to zero

In any case, we may consider some of the implications of this competition as far as they concern the excretion of potassium under ordinary conditions If it is assumed that the effects of alkalosis are produced through the mechanism involved in the competition then we must conclude that at least under these circumstances secretion contributes heavily to potassium excretion even though the excreted potassium does not exceed the amount filtered This is not a new concept but I believe the contributory evidence is rather convincing Perhaps even more important if we accept that acidosis per se tends to reduce potassium excretion then secretion must contribute an important part of even normal potassium excretion I have difficulty in conceiving this effect of alkalosis as being something suddenly turned on there having been no potassium secretion before with a normal acid base balance I prefer to think of it as a continuous process It is our feeling although there is no conclusive evidence that a large proportion if not all normal potassium excretion is attributable to tubular secretion

A few comments about the competition seem in order before we go on to somewhat different topics The competition is not on a one-to-one basis When carbonic anhydrase is inhibited the increase in bicarbonate excretion far exceeds the increase in potassium excretion plus decrease in titratable acidity This we presume indicates either that the turnover rate for hydrogen ions is greater than for potassium in the system involved in the competition or that under these circumstances the rate limiting reactions are no longer those involved in the competition Furthermore although the effect of #6063 on potassium excretion seems to be near maximal with a dose of 5 mg per kilo the effect on bicarbonate excretion seems to increase further with larger doses

Another point that should be mentioned is that when potassium secretion is inhibited with mercurial diuretics there is no effect on

acidification We interpret this to mean that it is the concentration of potassium at the site of competition which plays an important role, rather than the turnover of potassium On the other hand, we may presume that since the production of hydrogen ions is dependent upon metabolic activity, failure of production and failure of secretion will go hand in hand

Thorn Did you ever observe increased potassium excretion in dogs?

Berliner Yes, we have frequently, under certain circumstances, that is, when there is to start with a very low potassium excretion That, in large part, has been the cause of the controversy over the effects of mercury on potassium excretion(6,16) I should be glad to take that up later

The effects of #6063 have caused us to re examine the significance of the carbon dioxide tension of urine It will be recalled that Dr Pitts(5) found that in alkaline urines the CO_2 tension of urine was often very much higher than that of the arterial blood and higher than the CO_2 tension of the kidney itself might be assumed to be This observation has been confirmed by others, notably Ryberg(17), and the same interpretation accepted, that the addition of hydrogen ions by secretion in the distal tubule converts bicarbonate to carbonic acid In the absence of carbonic anhydrase in the fluid, the dehydration of the carbonic acid is sufficiently delayed to permit escape of the urine from the tubules before equilibration of the CO_2 occurs As collected from the bladder, this extra CO_2 appears as a marked elevation of CO_2 tension The presence of an elevated CO_2 tension in alkaline urines is taken as evidence that hydrogen ion continues to be secreted even in the presence of alkalosis We were, therefore, surprised to find that when the mechanism for secreting hydrogen ions had presumably been knocked out by #6063, the CO_2 tension of the urine was every bit as high as in urines of equivalent pH formed in alkalosis This could be interpreted as indicating that #6063 produced its effect by inhibiting some other mechanism for reabsorbing bicarbonate, but we thought we had better look into the question of what the CO_2 tension means

We considered other possible explanations of the elevated CO_2 tensions One of the earliest explanations suggested was that it is due to the mixture of urines of varying pH This is undoubtedly a factor in studies such as those of Mainzer(18) where urines were collected in the bladder overnight However, in our studies the urine was collected as quickly as it appeared in the bladder so that the total specimen on which the CO_2 tension was determined repre-

sented at most two to three minutes of urine flow and at a time when there was no appreciable over all change in the urine pH. Therefore it appeared that if there were any mixture of urines of different pH these urines must be being formed continuously and simultaneously.

On considering some method of exploring this possibility further we realized that if two solutions of different pH were mixed the resulting elevation of CO_2 tension would be highly dependent on the concentration of buffer. For instance if two solutions of bicarbonate and carbonic acid of equal CO_2 tension and of pH 7 and pH 8 respectively were mixed no change in CO_2 tension would result since there would be no hydrogen ion available from the

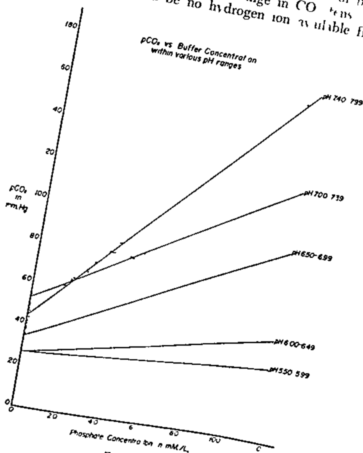


Figure 79

more acid solution to convert bicarbonate from the more alkaline to CO_2. However, if some other buffer were also present this would represent a source of hydrogen ion and the CO_2 tension would rise.

An investigation of the relationship between buffer concentration and CO_2 tension revealed that the two are indeed highly correlated, as shown in Figure 79. Even the most alkaline urines do not show much elevation of CO_2 tension unless there is an appreciable concentration of buffer present, and the elevation of CO_2 tension is more or less directly proportional to buffer concentration. This is a characteristic not only of alkaline urines but of urines of all pH's, although the slope is steeper and the greatest elevations of CO_2 tension are reached with the more alkaline urines.

Darrow: Are those urines as voided?

Berliner: Those are urines as collected from the bladder. What we do is put a catheter in the bladder, allow the catheter to fill with urine, attach a silicon coated syringe to the catheter, and withdraw urine as quickly as it appears in the bladder. We then disconnect the syringe, stopper it, and determine the pH and CO_2 content within a very short time. I shall come back to the method of estimating the CO_2 tensions shortly.

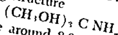
It can be seen from Figure 79 that the most alkaline urines do not show much elevation of CO_2 tension if there is not much buffer (phosphate) present and that as the phosphate concentration is raised the CO_2 tension goes up. We did not indicate which of these urines are which, but some were alkaline because the dog was alkalotic and some were alkaline because the dog was given #6063. It doesn't make any difference which way it is done.

It is also worthy of note that some of the CO_2 tensions are actually lower than that of the arterial plasma, and certainly considerably lower than that in the renal tissue. This is particularly true of the more acid urines. Dr. Pitts has observed the same thing(5). We do not think that this is due to loss of CO_2 from the urines or to any other experimental error; there is good reason to believe that this can occur under certain conditions when urines of different pH are mixed. This is particularly true when the urines are relatively acid, when there is a higher concentration of buffer in the more alkaline components of the mixture, and when the pK of the buffer is close to the pH of the urines involved.

I might say a word about how our CO_2 tensions are estimated. These CO_2 tensions have been calculated from the pH and CO_2 content using a pK' of 6.1 for carbonic acid and a uniform solubility coefficient of 0.0309. Some variation of results is to be expected

since it is improbable that these values really remain constant under the highly variable physical conditions encountered in urine. However, we have checked a number of our calculated estimates at both high and low levels against direct determinations by the Roughton Scholander syringe method of Riley (19) and the correspondence has been very good. In addition in Ryberg's studies (17) where all measurements were done by tonometric equilibration the data given for pH and CO_2 content yield calculated CO_2 tensions close to the determined values.

We were concerned that this might be interpreted not as an effect of buffer generally but as an effect of phosphate specifically. We tried to find some other buffer that would be effective between pH 7 and 8 and had quite a job. We finally tracked one down and examined its effect. This buffer is tris(hydroxymethyl) amino methane which has the following structure:



$$(CH_2OH)_3\, C\, NH_2$$

It has a pK somewhere around 8.0 or 8.1, can be given in fairly large amounts to the dog without apparent ill effect and is rapidly excreted in the urine. Its effects on CO_2 tension are quite similar to those of phosphate in regard to its pK, the concentrations obtained, etc.

How is this to be interpreted? We believe it to be a manifestation of the heterogeneity of the individual nephrons particularly with respect to the pH and buffer concentration of the urine they form. Differences in pH may arise from at least two factors: differences in the amount of acid added by any individual tubule and differences in the extent to which the urine is concentrated in any individual tubule, the CO_2 tension in any single tubule presumably remaining in equilibrium with that of the surrounding tissue. Variation in buffer concentration in the urine from different tubules will also be a factor in determining the final CO_2 tension, this again being subject to variation because of variable reabsorption of buffer and concentration to a variable extent by the reabsorption of water. Finally, there is a factor of interrelation between pH and buffer content since, in general, the pH of the urines from nephrons of higher buffer content will tend to remain closer to that of plasma.

When all these factors are taken together it seems entirely possible that the urine coming from various tubules may vary enough to account for the observed effects. We have carefully considered all the alternatives and have not been able to discover any other which will account for the relationship between CO_2 tension and buffer concentration. One other mechanism would produce a rel-

tionship, although a different one, between CO_2 tension and buffer concentration, namely, if there were secretion of alkali by the tubule in such a way that the hydroxyl ions might take up CO_2 from the tubule fluid to form bicarbonate In this case, however, one would expect the highest buffer content to be associated with a CO_2 tension merely approaching that of the surrounding tissue The elevation of CO_2 tension is, we believe, a reminder of the fact that there are two million or more units involved, subject to biological variation, and that the two kidneys are not one big nephron as we are so often given to thinking

Pitts Could I ask one thing? I don't understand that top curve in Figure 79 Does that mean that all of the urines having pH values between 7 4 and 7 9 were collected and their pCO_2 values plotted against –

Berliner – against the phosphate concentration in those urines? Yes

Pitts Were the experiments done in such a way that the alkalinity of the urine, whether induced by carbonic anhydrase inhibitors or by bicarbonate administration, increased with the phosphate concentration?

Berliner No that isn't true Most of these experiments were done in this way We produced alkalosis by giving an infusion of bicarbonate, keeping phosphate low, this accounts for the points at the lower left portion of the Figure Then we injected phosphate to raise the urinary buffer concentration As we did so the urines moved up and to the right along the curve Actually, the urines with the low pCO_2's have higher pH's than those up at the right with high buffer contents and pCO_2's

Perhaps Figure 80 might clarify the point This Figure includes our data from periods where we had measurements of pH, CO_2 content, and phosphate concentration The bicarbonate concentration has been plotted against pH throughout the observed range, roughly differentiating the urines with respect to phosphate concentration as follows the open triangles are urines with a phosphate concentration of less than 20 mM per L, the open circles those between 20 and 49, and the solid dots 50 mM per L or more To clarify the relationship, particularly at low pH, and to make the vertical displacement for equivalent multiples of pCO_2 change the same in all pH ranges, the data have been plotted on a semilogarithmic scale The lines represent CO_2 tensions of 30 and 50 mm of Hg respectively

It can be seen that the relationship between CO_2 tension and

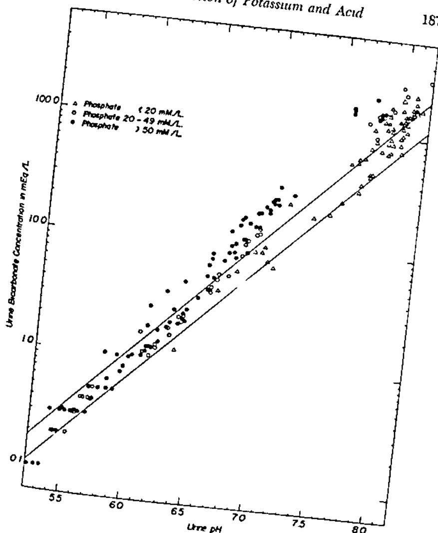


FIGURE 80

phosphate concentration holds roughly at any pH so that at each pH the bicarbonate may vary over a wide range as a result of the effect of buffer content on CO_2 tension

Pitts Not counting bicarbonate as a buffer?

Berliner Bicarbonate, for the purposes I am referring to here should not be considered a buffer

Pitts You mean it is not a good buffer?

Berliner I should have made this clear sooner. Where I have said buffer, I should have said buffer other than the bicarbonate carbonic acid system The reason for this is that bicarbonate cannot give up its hydrogen to convert other bicarbonate to CO_2 and water *Incidentally*, the theory *that the high* pCO_2 *is due to addition of hydrogen ions to urine containing bicarbonate with delayed release of* CO_2 would require just the opposite relationship In general, the higher the buffer concentration, the lower the expected CO_2 tension

I should like to call attention to one additional observation on the effect of #6063, namely, its quantitative effect on the excretion of bicarbonate You will recall the hypothesis that bicarbonate is reabsorbed in the proximal tubule in such a way that when the plasma bicarbonate is less than about 20 mEq per L, no appreciable amount is delivered to the distal tubule, all the bicarbonate being reabsorbed in the proximal segment As plasma bicarbonate is increased beyond this level increasing amounts are delivered to the distal segment which can reabsorb approximately 0 5 mEq per minute for each 100 ml of glomerular filtrate, the mechanism in this segment is the one which secretes hydrogen ion and requires the participation of carbonic anhydrase This mechanism is saturated at a plasma bicarbonate of about 25 mEq per L and above this the excess is excreted in the urine(5) According to this partition of bicarbonate reabsorption, if that portion dependent on carbonic anhydrase were to be knocked out, we would expect varying amounts of bicarbonate to appear in the urine as follows when the plasma bicarbonate is below about 20 mEq per L, there is no bicarbonate in the urine, when the bicarbonate is between 20 and 25 mEq per L, there is between 0 and 500 mEq per min, when plasma bicarbonate is above 25 mEq per L, there is an increment of bicarbonate excretion of about 500 mEq per min Table XI shows what actually is observed In this severely acidotic dog, when #6063 is injected the urine becomes alkaline despite a plasma bicarbonate well under 10, and appreciable amounts of bicarbonate appear in the urine at all levels of plasma bicarbonate Except for a somewhat lower fraction in the first two periods, probably because of a somewhat low filtration rate, approximately 40 per cent of the filtered bicarbonate is excreted at all levels of plasma bicarbonate

I offer no explanation for these findings They apparently require a drastic revision of the theory, just where, we are not yet prepared to say

Merrill How much of your drug appeared in the urine?

TABLE XI
Effect of #6063 on Excretion of HCO_3
Dog M 19 3 Kg

Time min	Urine Flow ml/min	Creatinine Clearance ml/min	Plasma HCO_3 μEq/ml	Filtered HCO_3 μEq/min	Excreted HCO_3 μEq/min	Excr/Filt HCO_3
0	Priming Creatinine 2 0 gm #6063 20 mg Kg					
1	Infusion Creatinine 13 mg min #6063 35 mg /Kg /hr in 0 85% NaCl at 5 2 ml min					
87	Replace 0 85 NaCl with 2 0% $NaHCO_3$					
87 109	2 45	49	9 7	481	86	18
109 127	6 70	52	12 2	649	214	33
127 147	7 35	57	12 9	764	302	40
147 167	7 21	56	15 0	869	382	44
167 188	6 34	56	17 6	1020	431	42
188 210	6 45	56	18 4	1081	496	46
210-232	6 97	56	(19 5)	(1142)	585	(51)
232 252	7 10	60	20 5	1280	596	47

Berliner This drug does not react with the reagents ordinarily used for the determination of sulfonamides so that we have not been able to measure it The people at American Cyanamid using the inhibition of carbonic anhydrase as an assay found a large part of the material excreted in active form in the urine*

Merrill Using a similar drug but by mouth we have found some of the effects you describe but we have been able to interpret them on the basis of the drug's action as an excreted anion We gave much higher doses in milligrams per kilo however and assumed complete absorption There is a lot of difference between the dose we used and the one you used so you could not interpret your data on those terms

Berliner For two reasons I do not believe we are dealing with that sort of phenomenon The drug you refer to is "Dirnate" is it not? "Dirnate" is a relatively strong acid #6063 has a pKa of 7 2 We have titrated a good many of the urines from dogs which had received #6063 and if there were any appreciable amount of it present that is as a factor in the total electrolyte composition of the urine we should have detected it in those titrations Further

* Roblin R O Jr Personal communication

more, the total dosage we used, from as little as 0 02 mM per kilo to about 0 5 mM per kilo, is very small compared to the enormous amounts of electrolyte excreted In general, we have been able to balance the measured cations and anions in the urine pretty well

Pitts Where would you put the site of action of #6063?

Berliner I am undecided Either the proximal mechanism by which bicarbonate is reabsorbed is also sensitive to these drugs or bicarbonate is secreted by the distal tubule I am not opposed to the latter idea, but we have no evidence for either alternative

Pitts Do you feel that you have attained a maximal effect with #6063?

Berliner I am not entirely certain but I think we are close With a dose of 100 mg per kilo per hour, we get 40 to 60 per cent of the filtered bicarbonate out in the urine We have not been able to influence that figure very much by the usual procedures, osmotic diuresis and so on We are certainly up to the point where we would have to give pretty large increments of drug dosage to increase the effect, and the solubility of the drug is sufficiently limited to make that a problem

Thorn Does #6063 have any initial effect on respiration?

Berliner We have thought that, at higher dosages, there might be some impairment of the release of CO_2 in the lungs It looked to us as though the dogs were hyperventilating, yet at the same time there was no fall, or even a slight rise, in arterial CO_2 tension We did not, however measure alveolar CO_2 tensions so we do not really know The impairment of CO_2 release is not very impressive at best

Barnett Do you get much change in plasma CO_2 content with #6063 in these experiments?

Berliner If acid is being infused, there is a relatively rapid drop in the pH and CO_2 content of plasma when #6063 is administered But otherwise there is not likely to be much change in these values during the relatively short period of observation involved in our experiments

Pitts With just #6063, what happens to the pCO_2 of the urine?

Berliner That depends on the phosphate content of the urine

Pitts Let's leave the phosphate out

Berliner With no buffer in th [illegible] comes alkaline with very little change in CO_2 t [illegible] of buffer in the urine, the CO [illegible]

I referred before to Rydberg's observations(17) You will recall that he too found that alkaline urines have high CO_2 tensions He

also noted that as the urine flow increased the CO_2 tension fe
Now, when the urine flow is increased the CO_2 tension should ri
if it is a matter of the delay in the dehydration of CO_2, because th
faster the urine is flowing, the easier it is for it to get out of –

Pitts No, not outside the time limits of dehydration in the tubule

Berliner If the urine flow gets way down it must take an appreciable time for the urine to get through the tubule We know that the so called delay time gets pretty long In any case, Ryberg's observations can be looked at in this way in general, the greater the urine flow the lower the buffer concentration is likely to be Our interpretation of his observation, and we have observed the same thing, is that it correlates quite well with buffer concentration Of course, the urine is never really free of buffer even when you cannot measure any phosphate or other known buffers in it There are some substances which will buffer it slightly at a pH above 7 where the creatinine is not a factor

Mudge By analogy, don't you think it is possible that bicarbonate is reabsorbed as a free acid rather than in an ionized form?

Berliner It is conceivable, although I would doubt it What would you do with the sodium that was originally with the bicarbonate ion? You must get the sodium back to reabsorb bicarbonate, otherwise, it's not really reabsorbing bicarbonate Now, if you want to think of reabsorbing the sodium and converting the bicarbonate to carbonic acid in the same process that is what we have been talking about all the time Alternatively, I concede the possibility that bicarbonate might be reabsorbed as such that is as bicarbonate ion, leaving its sodium behind and being replaced by some other anion

Darrow Is there anything in the data which excludes the bicarbonate being reabsorbed

Berliner No

Darrow You mean it is just an alternate hypothesis?

Berliner Yes, it is However, I don't think really good acidification an be accounted for by reabsorption of the bicarbonate because ere is not much bicarbonate left when there is good acid urine Part of the process of bicarbonate reabsorption might be something of the sort, that is, exchange for some other anion At least it is compatible with everything we know

Pitts If you consider the data of Walker, Bott, Oliver, and McDowell(20) which demonstrate an increase in the concentration of chloride in the first half of the proximal tubule and couple that with the facts that the urine remains isotonic with the plasma in

this segment and that the sodium concentration is unchanged, then bicarbonate must be rather considerably reabsorbed In the face of rather complete absorption of bicarbonate, I find it difficult to believe that pH doesn't change

Berliner How can you get a change?

Pitts How can you avoid getting it, if you absorb the bicarbonate nearly completely?

Berliner I think that there must be, under the conditions of their experiments, a change in the pH of the proximal tubule urine Whether that is due to reabsorption of bicarbonate by some anion transport mechanism or by some cation transport mechanism, that is, hydrogen ion secretion, I do not think we can say

Pitts You would have to say that sodium and bicarbonate went back in equivalent proportions

Berliner Well, of course, if you get the sodium back the way we presume it gets back in the distal tubule, you don't have to worry about the bicarbonate I would prefer to think of it that way, personally but that may not be correct

Thorn Have you given #6063 in man?

Berliner We have not, but other people are doing it

Thorn Do you think you would get any lead on mechanisms involved by analyzing the mineral changes in saliva rather than in urine?

Berliner I suspect that the degree to which this drug is concentrated in the kidney is very important, and that to get effects anywhere else you might have to give very large doses

Thorn You don't have to do that with adrenal hormones The mechanisms appear to be similar

Berliner But that presumably involves some specific system localized in the areas where its effects are observed Carbonic anhydrase, on the other hand, is pretty universally distributed and probably involved in a good many types of processes I would not want to try it on a patient first

Thorn That is what I mean Presumably, it is carried out on the dog

Darrow Have you done that in the absence of adrenals?

Berliner We haven't The rapidity with which the effect comes on is such that it would be inconceivable to me that it was anything but a direct effect

Thorn Sometimes, in a system like that, we obtain a more general reaction

Berliner The people who have tried doing this with the idea of inhibiting gastric acid secretion have never succeeded in making it work It has been calculated that the excess of carbonic anhydrase activity is such that before gastric acid secretion could be inhibited red cell carbonic anhydrase would probably be so completely knocked out as to cause severe respiratory distress

Mudge Is that with #6063 or with less potent inhibitors?

Berliner That is just in general based on the distribution of carbonic anhydrase activity

Darrow Have you tried it by injecting the stomach locally?

Berliner No

Binkley It has been tried in isolated preparations i e mouse omachs

Berliner Yes Carbonic anhydrase inhibitors do knock out acid secretion by the isolated frog gastric mucosa and by mouse or rat stomachs

Mudge One point may be of minor interest There are now several experimental procedures which stimulate potassium secretion Cellular dehydration and the infusion of potassium salts may act in the same manner by elevating intracellular potassium Inhibition of carbonic anhydrase has now been added to the list In addition Foulks and Gilman* have shown that the infusion of lithium salts rapidly stimulates the tubular secretion of potassium After Dr Berliner s paper appeared we quickly ran some experiments to find out whether lithium had an effect on carbonic anhydrase We used an enzyme preparation from human erythrocytes and were unable to demonstrate any effect of lithium It therefore acts by some other mechanism

Berliner But Foulks and Gilman did not as I recall measure the pH of the urine

Mudge No they did not determine the pH

Berliner I mentioned two years ago that maleic acid knocked out acidification We have since re examined our data and found that it showed the same effect on potassium excretion that we have found with #6063 At the time we did not pay much attention to it We have since investigated the effect of maleate on carbonic anhydrase and found that it has no activity whatsoever It must inhibit some other process involved in the production of hydrogen ions but what one we do not know Of course we cannot be certain that #6063 does nothing but inhibit carbonic anhydrase but its activity is so great and the dose required so small that it is difficult

* The work of Foulks and Gilman is as yet unpublished

to believe carbonic anhydrase is not involved Possibly we visualize its location in the wrong place Perhaps the carbonic anhydrase is important in the proximal rather than the distal tubule, or maybe in both, but I do not think we can throw out carbonic anhydrase

I should like Dr Bott to get us more data on what happens to pH in the mammalian proximal tubule I would like to think that it was constant under all these conditions, but there is not much evidence for it except for a few measurements of chloride and sodium which did not leave much room for bicarbonate Those experiments were all done under the same conditions, weren't they?

Bott Yes, the few that we have on that

Berliner We think possibly the frog may prove useful in answering this question and plan to investigate it

Pitts Dr Berliner, in connection with the effects of alkalosis and acidosis on the serum level and balance of potassium, I have been trying to tie together in my mind, in some rational way, your findings and those of Dr Darrow How far would this idea be out of line with reality, namely, that the effects of alkalosis are primarily to promote the elimination of potassium? The extracellular level of potassium would be lowered and since the equilibrium across the cell membrane would be disturbed, potassium would tend to be lost from the cells That would be described as a primary renal effect The effect of acidosis might not be in any sense a primary renal one, but rather a primary effect on the binding of potassium within cells In severe diabetic ketosis, serum potassium is normal or elevated slightly, potassium is lost from the cells, and the over-all potassium balance is negative, i e , potassium excretion is increased Essentially the same findings are observed in ammonium chloride acidosis, although the degree of negative potassium balance is less than in severe diabetic ketosis This is merely an attempt to correlate the fact that in severe diabetic or ammonium chloride acidosis, one does not have a low extracellular potassium although excessive potassium loss occurs, whereas in alkalosis one does I would associate the primary defect in potassium excretion with the kidneys, so far as alkalosis is concerned, but with abnormalities in the cellular mechanism for holding potasium in conditions of acidosis

Berliner I do not think we can overlook what happens in the cell, it certainly is an important factor But it has never appeared to us that the plasma potassium concentration had much to do with the rate of potassium excretion That is one of the striking things about it

Pitts Just how, in giving 5 or 10 grams of potassium by mouth

do you get an increase in excretion of potassium if you have no change in plasma concentration

Berliner We have thought that it was due to the uptake of potassium by the cells involved in its excretion

Pitts What causes the cells to take up potassium?

Berliner Its availability

Pitts You mean there is an infinitesimal rise in concentration?

Berliner The situation there is that you raise the plasma potassium so potassium goes into cells On the other hand if you raise the plasma potassium by taking potassium out of cells unless the cells in the kidney work in exactly the opposite direction from all the other cells — and I can t deny the possibility —

Pitts It would be my assumption that so far as the renal tubular cells are concerned they are operating in an entirely different region of the potassium spectrum

Berliner As I say I would not deny the possibility but I think that loss of anions say or whatever it is that would have to be inside cells with potassium is not unlikely in diabetic acidosis However I do not think it is a very likely explanation of the situation in ammonium chloride acidosis

Pitts Dr Guest(21) observed in ammonium chloride acidosis that there was hydrolysis of the organic phosphates within red cells in exactly the same fashion as occurs in diabetic acidosis Is that not true Dr Darrow?

Darrow Yes but Guest found a change in organic phosphates in red cells which quantitatively cannot be so very important

Pitts No but you might assume that the same factors hold in the red cell

Darrow Yes I think so but it is an assumption

Dr Berliner my original surmise concerning the role of the kidney in regulating acid base equilibrium in potassium depletion was that the tubular cells might be deficient in potassium We have analyses of the whole kidneys of rats in potassium depletion due to diets low in potassium and in response to injections of desoxycorticosterone acetate Compared to controls we cannot demonstrate a statistically significant difference in potassium content or in the relation of chloride to sodium That does not exclude a local change The data are analyzed and I hope to report them soon If you want to show a loss of potassium from cells you will probably have to select a part of the nephron for anal be difficult

Berliner I agree One thing about this ammonium chloride acidosis the observation is that the muscle potassium concentration is high

Pitts Not in ammonium chloride acidosis

Berliner Under the conditions of acidosis that Dr Darrow used in his rats This acidosis was produced not by increasing the chloride but by decreasing the sodium I think that brings it more into the category of changes that I have spoken about, namely, the situation where you are not going to change total electrolyte excretion greatly

Mudge To me, at least, the evidence does not indicate that the equilibrium across the muscle cell is dependent just on pH In renal tubular acidosis, there is a failure of the renal acidification mechanisms This is associated with a fairly marked systematic acidosis These patients are in negative potassium balance, and this is reflected in the muscles where intracellular potassium has been replaced by sodium Regardless of the extent to which the muscle composition might depend on plasma pH, it is certainly rue that in this instance the equilibrium at the muscle level is etermined by renal function

Darrow I do not believe we are in a position to say what the iscle equilibrium is with respect to the extracellular fluids because ry time one quotes an observation of the possible relationships, kidney raises its ugly calyx into the picture and possibly alters environment, and one does not know that the equilibrium that served is one which would occur without the kidneys It seems to me that we have to take the kidney out of the picture in order to determine what the equilibrium between cells and extracellular fluids really is At present we have a set of observations which can be explained by the kidneys alone, by an equilibrium between extracellular fluids and cells, with the kidneys playing a secondary role or, as is more likely a combination of both processes We just do not know the exact local effect on cellular composition produced by variations in extracellular sodium, potassium, or chloride, or anything else Future observations must exclude the effect of the kidney

I believe the buffers of the body as a whole remain relatively constant and that acid base equilibrium and distribution of water are a result of the content of these crucial ions The distributions of sodium chloride and potassium are maintained between extracellular and intracellular fluids by osmotic forces and vital processes The amounts of Na, Cl and K in the body determine the bicar-

bonate concentration of serum, depending on the buffers of the body as a whole and the distribution of Na between intracellular and extracellular fluids

The factors controlling the balance of potassium in the body were stated by Dr Pitts much as I would state them tentatively, that is, in acidosis, urinary loss of potassium is dependent on the fact that, when a large load of anions is being excreted, sodium alone may be used at first to excrete the acid but as deficits of sodium become appreciable, potassium is also excreted In alkalosis there is apparently a tendency to excrete excessive amounts of potassium, at least until potassium deficits are considerable Then potassium may be saved or at least appear only at low concentrations in the urine Doubtless, exceptions may be found to these rules In diabetic acidosis, there is apparently a release of potassium from the cells not dependent solely on urinary excretion of anions

Berliner If we leave diabetic acidosis out of it for the moment the other observations on muscle potassium seem to make sense In the intact animal whose kidneys are working one way or another either not so well, as in the cases to which Dr Mudge referred or normally, as we presume Dr Darrow's rats behaved the amount of potassium found in the muscle correlates quite well with the amount of potassium in the body and the amount available for the muscle to take up

Darrow Yes, and it leaves you uninformed with respect to the obligatory relationship between cell composition and extracellular concentrations The findings merely illustrate what the body does with a given overall content of water and electrolyte

Pitts What is the action of DCA? Where do you put that?

Berliner I would not want to commit myself I have a hunch that your observation as to the effect of DCA on acidification(22) plus the known effects of DCA on potassium excretion, suggest that it may well be this mechanism for exchanging both for sodium that is involved It is known that if an animal is not given sodium, it does not make any difference how much adrenal steroid he is given, he will not lose potassium If he is given some anion with which to excrete the potassium then he will lose potassium in the urine

Pitts Is that absolute, or is it true only after he has lost some potassium?

Berliner This is right from the start That observation was made

a considerable extent upon the condition when the hormone treatment is initiated. If you begin with a patient with a high serum potassium and administer DCA, he will certainly lose a relatively large quantity of potassium, whereas, if you start with a patient who has not retained much potassium beforehand, the hormone administration does not stimulate, or result in, the excretion of large quantities of potassium.

Berliner: Is that true whether or not the patient has any electrolyte intake? I think, in this instance, that is the critical point. There is no doubt that a patient with adrenal insufficiency, given DCA will lose potassium, at least down to the normal level if he has the intake a patient ordinarily gets. But these experiments were done with restricted diets and there was no substantial loss.

Thorn: These observations are all in the first twenty-four hours, and the effect which I have described may occur in the first few hours.

Darrow: But what it amounts to is that, over a long period of time, depletion of body potassium does not occur without diets containing considerable sodium chloride. However, there is another type of loss of potassium which is illustrated, as you stated previously by diabetes. As a patient goes into coma, the cells release potassium (I do not know of any better way of expressing it), and the release is apparently associated with a disturbance in a metabolic process. Potassium is apparently also released in an analogous manner as in anoxia and shock, and sodium has been shown to enter the cells under these circumstances. I have often been asked how I explained the observation of high serum concentration of potassium when the cells are known to be deficient in this ion. I have thought that the conditions in which these observations were made were always associated with a disturbance in cellular metabolism such as occurs in diabetic coma, the dehydration of infantile diarrhea, and shock. Under these circumstances, the cells are either unable to transport potassium back into the cells or to bind potassium. I do not know which mechanism is involved or whether either of the expressions has true chemical meaning. But I think the phenomena are more complicated than just a membrane equilibrium. It is also a metabolic equilibrium or steady-state.

Berliner: I think everyone would agree that it takes metabolic activity to keep potassium in the cells.

Darrow: And knocking out just one metabolic function may alter the equilibrium although the cells are still able to function.

Berliner: That is true, but I think we have to say that in any

condition in which the loss of potassium from the cell is primary you must find a high plasma potassium

Darrow What do you mean by primary?

Berliner I mean if the trouble starts in the cells and they lose potassium then I think you must find a high plasma potassium

Darrow I would have called the loss from the body as a whole as a result of excessive excretion or deficient intake a primary potassium deficit But your definition is all right if understood as such

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ENZYMATIC PROCESSES IN TUBULAR SECRETORY TRANSPORT*

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BEFORE REPORTING ON recent progress along our main line of investigation, I should like to summarize very briefly our present views concerning one kidney enzyme which was discussed at considerable length in the first of these conferences(1). It will be recalled that two years ago Dr. Binkley told about his interesting observations on a lipoprotein complex which he had isolated from pig kidney. This material constituted some 30 per cent of the kidney protein by weight and had many of the properties of an ion exchange resin of the carboxylic type. The only enzymatic activity apparent in the preparation was that of a glutaminase. Dr. Binkley was impressed by the quantity of this lipoprotein in kidney and suggested that essentially all of the active tubular reabsorptive and secretory processes involve ion exchange mechanisms and depend on the hydrolysis of glutamine as the primary source of energy.

During the past two years, we have made a few observations on this system in the rabbit kidney and have reached certain general conclusions. As a starting material, we have used the "cyclophorase" enzyme preparation of Green, Loomis, and Auerbach(2). This consists of the washed nuclear and mitochondrial particles of kidney homogenates, and constitutes, in rabbit kidney, about 30 per cent of the original tissue. One finds within these particles (i.e., specifically in the mitochondria) all of the enzymes which implement the citric acid cycle, which catalyze the complete combustion of fatty acids and certain amino acids and the cytochrome-cytochrome oxidase system of electron transport. We have since found that the cyclophorase preparation is rich in glutaminase activity.

When this material is treated successively by a series of procedures, such as freezing and thawing, lyophilization, increasing temperatures, etc., the stepwise disappearance of the various enzymatic activities is observed until the only remaining one is that of the so-called "glutaminase." This is present at its original level and

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is indeed an extraordinarily hardy enzyme We have attempted without success to isolate the glutaminase from the rest of the complex The cyclophorase preparation can be ground with desoxycholate to yield a very thick viscous material This is the procedure used by others to solubilize cytochrome oxidase(3) Subsequent treatment of the viscous material with streptococcal desoxyribonuclease depolymerizes the nucleic acids and yields after dialysis an almost water clear solution which contains all of the original glutaminase activity When one applies the conventional protein fractionation procedures (alcohol ammonium sulfate iso electric precipitation etc) no appreciable purification can be achieved Very low concentrations of alcohol or ammonium sulfate precipitate the whole protein complex No further attempts along these lines are contemplated

The foregoing observations deal with a preparation which is presumably very similar to that of Dr Binkley The abundant lipoprotein complex isolated from kidney does normally house a great variety of important enzymatic activities Certain preparative procedures may destroy all but the hardiest of these without altering the gross appearance or quantity of the material The simple demonstration of glutaminase activity in the final preparation therefore should not lead us to overemphasize the importance of this enzyme to the exclusion of others or result necessarily in the belief that the hydrolysis of glutamine provides the major portion of the energy utilized in tubular transport

I should like to pause at this point to ask Dr Binkley for his comments

Binkley I have nothing special to say on that We are not certain that the enzyme makes up 30 per cent of the kidney protein but I would say it is extremely high The point is that it does not occur in every tissue in mitochondrial fractions if you want to call them mitochondrial fractions except in the intestine or the kidney We do not feel that we are dealing with mitochondria in our preparations The so called mitochondrial fraction after all is nothing but a collection of all the debris of the cell that is the insoluble debris of the cell

Taggart I do not agree From the standpoint of integrated enzyme systems it gives evidence of being a very real entity

Oliver Especially is this true in the kidney One can get particulate matter from a kidney brei by means of differential centrifugation which has all the morphological characteristics of mito-

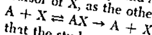
chondria and which stains with Janus green and is not contaminated with debris. This is routine procedure in our laboratory.

Binkley: In that sense, yes, but the insoluble material from the kidney need not necessarily be called mitochondrial fractions. We have used the sucrose purifications and other procedures that eliminate Janus green staining activity.

Taggart: I think we should get away from the idea that the glutaminase is important simply because of its apparent bulk. At the present time, we have no real measure of its quantity.

Binkley: The point I made and would still make is that this is the one activity that is present in the kidney and intestine exclusively, in large amounts.

Taggart: I should like to confine my further remarks to one tubular transport mechanism, namely that which is responsible for tubular excretion of para aminohippurate (PAH), diodrast, penicillin, and, probably, phenol red. Our interests are primarily the biochemical events which underlie the active tubular transport of these compounds. Expressed in the simplest terms, it may be said that A (the compound being transferred) must react with some cellular component, X, to form an intermediate AX. The compound AX must in turn be capable of undergoing dissociation or cleavage to give rise to the parent compound A as one of its products and either X, or a precursor of X, as the other product.



$$A + X \rightleftharpoons AX \rightarrow A + X$$

It may be recalled that the studies presented here two years ago appeared to have established the following points: (*a*) The tubular excretion of PAH, diodrast, and phenol red operates at the expense of phosphate bond energy generated by the aerobic oxidative reactions of the citric acid cycle. Since there is no reason for believing that the transported compounds are phosphorylated, it is assumed that the phosphate bond energy is utilized for priming or activating some cellular component of the system. (*b*) The rate of PAH transport can be strikingly increased, i.e., almost doubled either *in vitro* or *in vivo*, by acetate and certain immediate precursors of acetate. (*c*) PAH transport is markedly depressed by the dicarboxylic acids of the citric acid cycle, by those amino acids which feed directly into the cycle, and by the fatty acids of C_8 to C_{12} chain length. (*d*) These stimulatory and depressant effects occur independently of predictable changes in the rate of cellular respiration. For example, α-ketoglutarate and succinate stimulate the respiration of kidney slices as much or more than does acetate but nevertheless are potent inhibitors of PAH transport. It follows

therefore, that the stimulatory effect of acetate is not attributable to a simple speeding up of the metabolic machinery Finally, I should like to emphasize that the concentrations of acetate or α-ketoglutarate needed to produce the maximal effects are rather low, somewhere in the range of 2 to 3 mM per liter

During the past two years many additional known metabolic intermediates have been tested for their effects on PAH transport in kidney slices It still seems safe to conclude that acetate, or some product derived therefrom, possesses a unique stimulatory action The inhibitory compounds are many and quite diverse in their chemical structures We have, of course, wondered why a readily oxidized substrate such as α ketoglutarate should be a potent inhibitor It was suggested two years ago that a speeding up of the citric acid cycle by α ketoglutarate might deplete the available supply of essential acetyl groups through the formation of tricarboxylic acids This was never an attractive hypothesis, for certain members of the cycle are not inhibitory In addition, α-ketoglutarate inhibition cannot be completely reversed by the addition of an excess of acetate A second suggestion was that α ketoglutarate might be competing with PAH for accumulation within the slice However we have never been able to demonstrate any net accumulation of α ketoglutarate

We examined the series of homologous straight chain dicarboxylic acids for their effects on both cellular respiration and PAH transport The results were as follows 0 01 M oxalate or malonate had little effect on either respiration or transport Succinate, as usual stimulated respiration and inhibited transport The most interesting results were those obtained with glutarate and adipate Both of these compounds appear to be metabolically inert insofar as respiration is concerned, but both proved to be potent inhibitors of PAH transport When glutarate was examined over a wide range of concentrations, its pattern of inhibition was found to be very similar to that of α ketoglutarate Consequently, we are probably in error when we attempt to explain the inhibitory effect of α ketoglutarate in terms of its known metabolic fate

When one reviews the long list of inhibitors of PAH transport it is found that they have only one apparent structural feature in common, namely, the carboxyl group It is of interest that the various compounds which appear to share the tubular secretory mechanism with PAH are also all carboxylic acids Thus, we are inclined to concentrate our attention on the carboxyl group as the one through which the transported compound can interact with

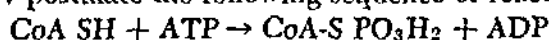
some cellular component Studies were undertaken to establish the carboxyl as the reactive group in one representative transported compound, PAH We already know that the p amino group is an unlikely site Both hippurate and para acetylaminohippurate are actively excreted by the tubule A second possible site for interaction is the amide nitrogen, $NH_2 C_6H_4 CO NH CH_2 COOH$ This now seems to have been adequately excluded A new compound, which might be called N-methyl PAH was synthesized, with sarcosine replacing the glycine moiety This compound was found to be actively excreted by the tubule at a rate only slightly less than that of PAH The methyl substituted PAH was recovered from the urine and identified as such

A third possibility suggested from time to time is the following (*a*) the hydrolysis of PAH to PAB and glycine and (*b*) subsequent reconjugation The first reaction is not known to occur in the kidney (4) but the second has been studied extensively by Cohen and McGil The synthesis of the peptide-like bond of PAH requires phosphate bond energy in biological systems More pertinent to the present discussion is the finding of Karl Beyer and his associates that Carinamide is an inhibitor of this reaction(5) Carinamide and its congener, Benemid, were originally introduced by Beyer as effective agents for delaying the renal excretion of penicillin and have since been found to depress the tubular excretion of PAH and phenol red as well If the mode of action of Carinamide is the same in all, the interference with phenol red transport certainly indicates that the conjugation reaction is not involved However, we have excluded this reaction in relation to PAH transport in another manner Para-aminohippurate was synthesized with C^{14} labeling in the carboxyl group This was infused slowly into a dog and subsequently recovered from the urine Since the filtration fraction in this animal was approximately 30 per cent it follows that about 70 per cent of the recovered PAH had been transported across the tubular epithelium In the event of hydrolysis and reconjugation during transport, the C^{14} in the carboxyl group should be diluted by the incorporation of unlabeled glycine from the metabolic pool Such was not the case the original PAH had a count of 493 ± 4 per minute and that recovered from the urine had a count of 490 ± 4

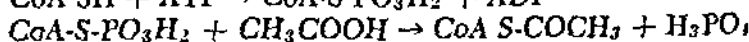
The foregoing experiments leave us with the carboxyl as the most probable reactive group In any speculative attempts to formulate a carboxyl mechanism related to tubular transport the following points should be borne in mind First the utilization of phosphate

bond energy must be implicated Second, the reactions involved may be expected to be associated with the enzymatic activities of the mitochondrion, and, lastly, certain interdigitations with acetate metabolism should be apparent It has been our belief that a clarification of the process by which acetate is "activated" might provide us with a useful working hypothesis The so called "active acetate" is that which participates in the acetylation of choline or sulfanilamide, in the condensation of two C_2 units to form acetoacetate, and in the condensation of acetate with oxalacetate to form citrate Some years ago, Lipmann proposed acetyl phosphate as the active C_2 unit but he and others later obtained abundant evidence opposing this view

The exact nature of "active acetate" remained one of the outstanding enigmas of modern biochemistry until very recently It now appears likely that Lynen and his associates in Munich have provided the answer(6) It has been known for several years that coenzyme A (co acetylase) participates in the previously mentioned condensation reactions involving acetate The complete structure of this coenzyme has not yet been determined, but it is known to contain adenine ribose, phosphoric acid, pantothenic acid, β mercaptoethylamine (decarboxylated cysteine), and probably glutamic acid(7) Lynen and Reichert have recently isolated from baker's yeast a form of CoA which can replace acetate + ATP + CoA in Lipmann's sulfanilamide acetylation system(6) Chemical studies indicate that the active component is the acetyl mercaptan of CoA They postulate the following sequence of reactions in its formation



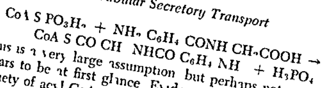
$$CoA\ SH + ATP \rightarrow CoA\text{-}S\ PO_3H_2 + ADP$$



$$CoA\text{-}S\text{-}PO_3H_2 + CH_3COOH \rightarrow CoA\ S\text{-}COCH_3 + H_3PO_4$$

Thus, the first reaction is the phosphorylation of CoA by adenosinetriphosphate, and the second is an exchange reaction in which acetate replaces the phosphate The acyl mercaptan may be regarded as possessing an energy rich bond comparable with that in an acyl phosphate

This scheme does appear to provide us with a basis for certain reasonable speculations concerning the tubular secretory mechanism We can assume for the moment that phosphate bond energy is utilized in the phosphorylation of CoA and that the phosphorylated CoA represents the activated cellular component of the transport system The next step involves the assumption that various carboxylic acids other than acetate may exchange with phosphate to yield the corresponding acyl mercaptans of CoA for example in the case of PAH



$$CoA\ S\ PO_3H_2 + NH_2\ C_6H_4\ CONH\ CH_2COOH \rightarrow$$
$$CoA\ S\ CO\ CH\ \ NHCO\ C_6H_4\ NH\ \ + H_3PO_4$$

This is a very large assumption but perhaps not as daring as it appears to be at first glance Evidence indicating the existence of a variety of acyl CoA s is already forthcoming from several laboratories Korey de Braganza and Nachmansohn have reported the esterification of glutamate with choline in the choline acetylase system of squid ganglia(8) By analogy with the acetylation system glutamyl CoA would be the presumed intermediate Second recent studies by Sanadi and Littlefield in Green s laboratory have demonstrated succinyl CoA as an intermediate in the oxidation of α ketoglutarate to succinate in a preparation of pigeon breast muscle(9) Shemin and Wittenberg have suggested succinyl CoA as an important intermediate in porphyrin synthesis(10) Finally Chantrenne has recently shown that the synthesis of hippuric acid from benzoic acid and glycine in rat liver is a CoA dependent reaction(11) The evidence now available points to benzoyl CoA as the probable intermediate in hippuric acid synthesis(5)

The last finding has led us to a reconsideration of the inhibitory activities of Carinamide and Benemid One may ask whether these agents specifically inhibit the hippuric acid conjugase system or whether they act as inhibitors of a variety of CoA enzymes Dr Joseph Stern at our request has examined Benemid for its effect on citrate synthesis and has found it to be an inhibitor Moreover he was able to demonstrate that the inhibition is located at the level of acetate activation rather than in the final condensation with oxalacetate Similarly we have found Benemid to be an inhibitor of the sulfanilamide acetylation system Diodrast is another though less active inhibitor of sulfanilamide acetylation

On the basis of these scattered observations we are inclined to believe that Benemid may be capable of occupying the thiol group of CoA and that it acts as an inhibitor of secretory transport by displacing PAH diodrast etc Such an explanation would be very much in accord with that originally proposed by Beyer namely that Carinamide occupies the active group of an essential enzyme of the transport system and by being refractory to excretion results in a so called renal blockade (12) Many of the other carboxylic acids which are inhibitors of this transport system may act in a similar manner The stimulatory effect of acetate remains something of a puzzle I suppose the most likely explanation would be an obligatory exchange reaction between acetyl CoA and PAH

I need not emphasize that much of this discussion has been purely speculative. Most of the evidence cited is admittedly indirect and circumstantial. However, one can construct from the available data a fairly attractive working hypothesis. The field of coenzyme A metabolism will undoubtedly continue to grow rapidly in the years immediately ahead. We hope that this growth will make possible a more direct approach to the problems of renal tubular transport.

Berliner: Do you think the sulfonic acid group might act in the same way?

Taggart: All of our evidence at the present time indicates that phenol red and PAH are handled by the same transport mechanism. If there is any validity to the proposed scheme, then I suppose one must presume that an interaction can occur between the sulfonic acid group and CoA to give an S-S bond.

There is one additional point which I should like to make. The tubular secretory transport mechanism handles the various carboxylic acids at rates which are quite independent of their apparent dissociation constants. This is not an uncommon feature of certain enzymes with rather broad substrate specificities; for example, d-amino acid oxidase oxidizes the various α-amino acids of the d configuration at widely differing rates and these rates are independent of pK.

Forster: Recent observations by Puck, Wasserman and Fishman(18) on isolated renal tubules indicate that the mechanism by which phenol red is transported across the cell may be broken down into two steps. Potassium is required by the mechanism which first binds dye in the tubule cell (step one). Calcium is not necessary for this first step but is required for moving the dye from the cell into the lumen (step two). Hence in the absence of calcium no dye can be carried over into the lumen, but since step one can still function, dye will accumulate in the cells to a concentration determined by the chemical potential which step one acting alone can bring to bear. With a balanced salt solution no dye accumulates within the transporting cells, but with high potassium and no calcium intracellular concentrations as high as 1000 mgm. per cent are achieved when the concentration of phenol red in the oxygenated solution is 1 mgm. per cent. The first step can be inhibited by such competitors for the phenol red transport system as para-aminohippurate and diodrast.

With a salt solution balanced with respect to potassium and calcium, phenol red is so rapidly transported across the cells in an isolated renal tubule preparation that there is no visible accumula-

tion within the cell, even when concentrations have been ... in the tubular lumen 6000 times ... bath ...

... accumulates in the ... maximal concentration of 1000 mgm per cent when the dye concentration is 1 mgm per cent in the sustaining solution This intracellular concentration can be blocked by the introduction of diodrast or para-aminohippurate The conclusion is reached, then, that there are at least two steps involved in cellular transfer, the first one of which, the passage of the dye into the cell, requires potassium, and the second one, the movement from the cell into the lumen, requires calcium

Mudge What happens if the potassium is lowered?

Forster Nothing, because there is no initial movement of phenol red into the cell hence, no concentration of dye is possible in the cell or in the lumen

Taggart I am most worried about the abnormal conditions in which accumulation is observed within the cells One does not know just what this type of intracellular accumulation has to do with transport You will recall that we observed the same sort of thing with fish tubules in the presence of iodoacetate I wonder whether certain constituents of the cell might not be breaking down to yield products with an affinity for phenol red If so, then the dye would be moving in accordance with the chemical potential and not against it

Forster That is true but the interesting point is that the first step is a more or less specific one I was quite impressed by the observation that it could be inhibited by the presence of such transport competitors as para aminohippurate and diodrast

Berliner Did they try other acids of similar pK that are not actively transported?

Forster I believe that no such observations were made

Berliner They did not try to see if this was simply a nonspecific displacement by an acid of a known pK?

Forster Not to my knowledge

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